A STUDY OF TRUCUT BIOPSIES OF HEPATIC LESIONS WITH SPECIAL REFERENCE TO IMMUNOHISTOCHEMISTRY AND SPECIAL STAIN

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the requirements for the award of

M.D. DEGREE

IN

PATHOLOGY – BRANCH III

APRIL 2013

Declaration

DECLARATION

I hereby declare that the dissertation entitled **"A STUDY OF TRUCUT BIOPSIES OF HEPATIC LESIONS WITH SPECIAL REFERENCE TO IMMUNOHISTOCHEMISTRY AND SPECIAL STAIN"** is a bonafide research work done by me at **Coimbatore Medical College and Hospital, Coimbatore** during the period from July 2011 - September 2012 under the guidance and supervision of **Dr. Dr. ARJUNAN, M.D.,** Professor, Coimbatore Medical College and Hospital, Coimbatore. This dissertation is submitted to **The Tamilnadu Dr. M.G.R. Medical University,** towards partial fulfillment of the requirement for the award of **M.D., Degree (Branch III)** in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

Place: Coimbatore **Dr. N.N. VEENAA**
Date :

Certificate

CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY OF TRUCUT BIOPSIES OF HEPATIC LESIONS WITH SPECIAL REFERENCE TO IMMUNOHISTOCHEMISTRY AND SPECIAL STAIN"** is a record of bonafide work done by **DR. N.N. VEENAA**, Post Graduate Student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore, under the supervision of **Dr. M. MURTHY, M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College and Hospital, and under the guidance of **Dr. ARJUNAN, M.D.,** Professor, Coimbatore Medical College and Hospital, in partial fulfilment of the requirement of the regulations of the Tamilnadu Dr. M.G.R Medical University towards the award of M.D. Degree (Branch III) in Pathology.

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Acknowledgement

ACKNOWLEDGEMENT

I would like, at the very outset, thank the Almighty who has created me for the special purpose of serving humanity through the most noble profession.

I gratefully acknowledge the continuous support rendered by the Dean **Dr. R.VIMALA, M.D.,** Coimbatore Medical College Hospital, Coimbatore.

I would like to express my deep and sincere gratitude to **DR. M. MURTHY,M.D.,** Professor and HOD, Department of Pathology, whose commendable encouragement and personal guidance have provided a good basis for the thesis.

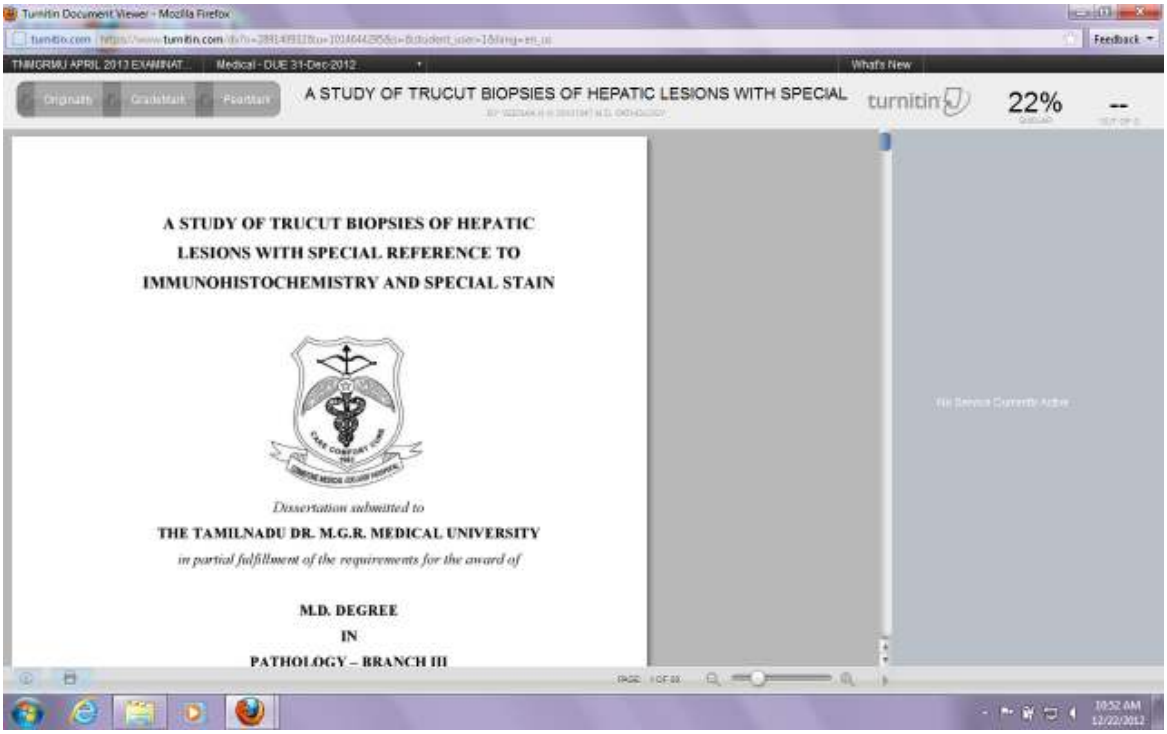
I am heartily thankful to my guide **DR. A.ARJUNAN, M.D.,** Professor, Department of Pathology for igniting a special interest on one of the most needed areas to be focussed in the current medical scenario.

My sincere gratitude goes to all my assistant professors and technical staff of the Department of Pathology whose good will and support has enabled me to complete the thesis to satisfaction.

Besides the expertise of my Professors, it is the co-operation of the patients that has helped me to arrive at accurate diagnostic

methodologies. So, it is my duty to record my humble gratitude for them as well.

My family has been a patient and all-round support system providing me with the enthusiasm and energy that was demanded and I am immensely thankful to them.



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Submission time 13-Dec-2012 12:55PM

Total words 11908

First 100 words of your submission

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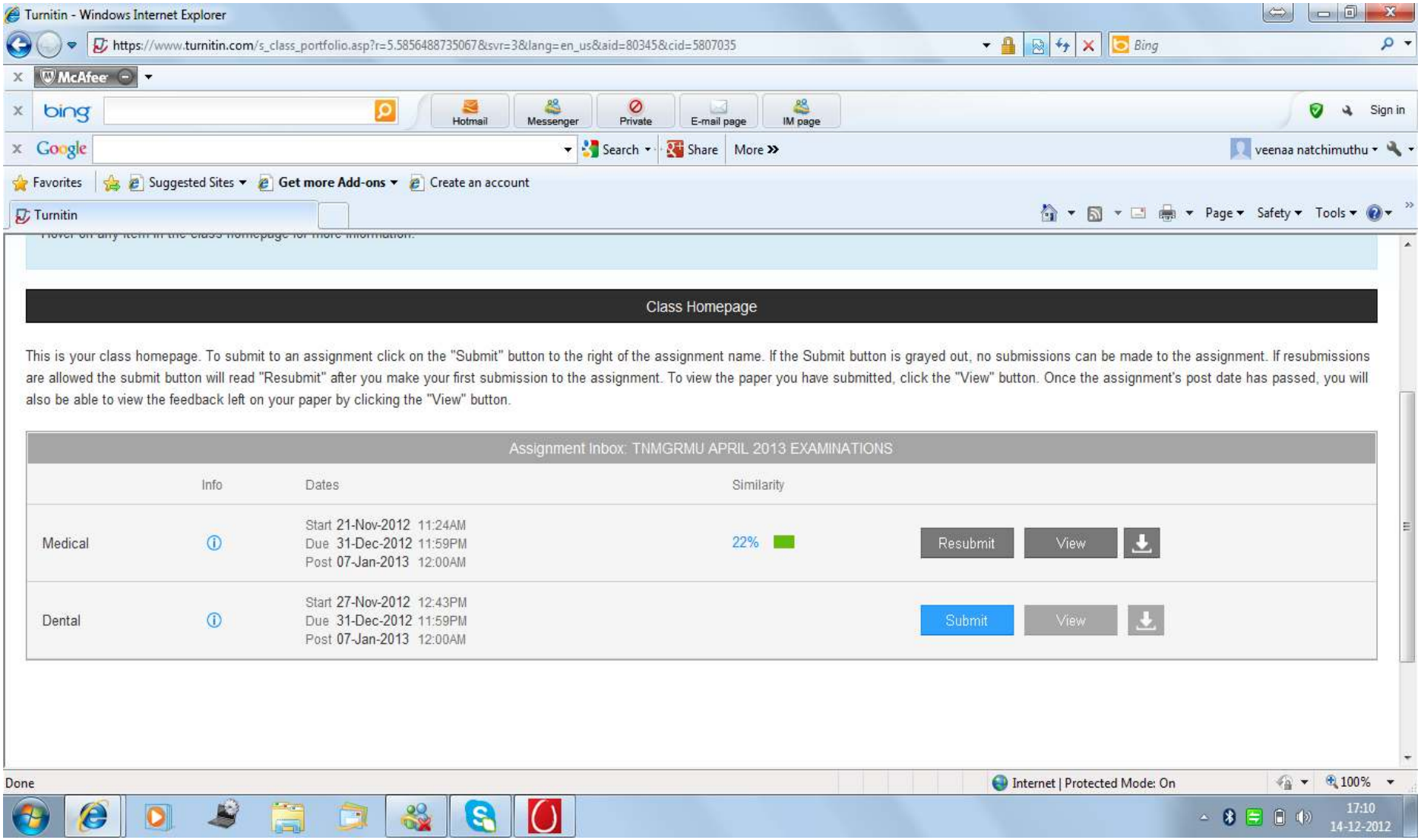
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LIST OF ABBREVIATIONS

HCC – Hepatocellular carcinoma

AFP - Alpha Feto-protein

P-CEA – Polyclonal Carcino Embryonic Antigen

m – CEA – Monoclonal Carcino Embryonic Antigen

CK – Cytokeratin

Contents

CONTENTS

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOUR PLATES

S.NO.	TITLE
1.	Hepatocellular carcinoma in low power
2.	Hepatocellular carcinoma in high power
3.	Immunohistochemical expression of AFP
4.	Immunohistochemical expression of Hep Par 1
5.	Liver biopsy with hepatocellular carcinoma in high power
6.	Hepatocellular carcinoma showing positivity for Hep Par 1
7.	Hepatocellular carcinoma showing positivity for AFP
8.	Hepatocellular carcinoma in low power
9.	Hepatocellular carcinoma in high power
10.	Hep Par 1 expression in hepatocellular carcinoma
11.	AFP expression in hepatocellular carcinoma
12.	Metastatic carcinomatous deposits in liver
13.	CK 7 expression in metastatic carcinoma of liver
14.	Liver biopsy showing hepatocellular carcinoma
15.	Expression of AFP in hepatocellular carcinoma
16.	Reticulin stain in hepatocellular carcinoma

Introduction

INTRODUCTION

Hepatocellular carcinoma is one of the commonest malignancies that account to about half a million deaths yearly. It ranks in the fifth place globally. 80% of these cases are seen in the Asian- Pacific region. Less than 20% of hepatocellular carcinomas get the appropriate treatment as surgical resection as therapy when they are diagnosed. It is purely due to the advanced stage of the disease at the time of diagnosis.

The risk factors of this cancer are well defined. Worldwide, about 80% of cases are due to cirrhotic livers. In Asia and Africa it is hepatitis B virus infection; as it is an oncogenic virus, it can cause cancer in the absence of cirrhosis.

In countries like Europe, Japan and United States, the bulk of the infection is due to hepatitis C than B because of the vaccination given for newborns.

Immunohistochemistry may help distinguishing hepatocellular carcinoma and its mimics. The challenges are attributed as follows:

1) Hepatic stem cell lineage can give rise to any neoplasm.
2) Nodular lesions of liver.
3) Metastatic tumours of liver.
4) Serum Alpha Fetoprotein has its own limitations.

The pivotal role of immunohistochemistry is in differentiating benign nodular lesions from reactive conditions, benign nodular lesions from well differentiated hepatocellular carcinoma, cholangiocarcinoma from metastasis and poorly differentiated tumours of hepatic origin and to ascertain the origin of the tumour whether primary or secondary.

Alpha Fetoprotein is the protein that determines the histogenesis of the tumour from the liver.

Bile canaliculi are stained by p-CEA and CD10.

Hep Par 1 is a hepatocyte marker.

CAM 5.2, Cytokeratin 8 and Cytokeratin 18 stain mature hepatocytes and nodules.

Cytokeratin 7, Cytokeratin 19, Cytokeratin 20 and Cytokeratin AE1/AE3 are absent.

Sinusoids take up CD34.

Alpha Fetoprotein, p-CEA, CD10 and CD34 are the primary panel markers to tell that the malignant nodule is hepatocyte in origin.

If histogenesis is questioned, then Hep Par 1 and Cytokeratin come into play.

The purpose of this study is to examine the usefulness of immunohistochemistry in differentiating primary from metastatic neoplasms of liver and to evaluate the role of reticulin stain to differentiate benign from malignant lesions of liver.

Aim of the Study

AIM OF THE STUDY

1) To study the role of immunohistochemistry in differentiating primary from metastatic neoplasms of liver.

2) To evaluate the usefulness of reticulin in differentiating benign from malignant lesions of liver.

Review of Literature

REVIEW OF LITERATURE

Hepatocellular carcinoma is a primary malignant tumour arising in liver, leading to mortality within a year. It commonly arises in a cirrhotic liver after a primary insult 20 to 30 years ago. But exceptions are noted where 25% of patients have no previous history or risk factors leading to cirrhosis.

After the introduction of vaccination for hepatitis B infection, screening and treatment for virus C infection decrease in hepatic lesions due to alcohol, cancer death due to hepatocellular carcinoma has gone down.

As the latency period from the damage caused to hepatocytes to the development of hepatocellular carcinoma is very prolonged, it takes years for the incidence of hepatocellular carcinoma to decrease after the usage of these interventions[1].

EPIDEMIOLOGY

Hepatocellular carcinoma accounts for 2% of all malignancies; since 1980s, the incidence has increased. There is increase in two fold in age adjusted incidence rate before one decade.

Annually there are 560,000 new cases diagnosed. It is the eighth most common cancer occurring in women. The prevalence of hepatitis B and C infections is directly proportional to the incidence of hepatocellular carcinoma.

In Asia and Africa, there is a high incidence of hepatocellular carcinoma as 120 cases per 100,000 population[1]. These high rates are due to infectious hepatitis.

RACE

Asians are the most common victims of hepatocellular carcinoma probably due to childhood infections with hepatitis B. As a result of implementation of vaccination for hepatitis B in many Asian countries, there is a fall in number of cases of primary liver malignancies.

SEX

Hepatocellular carcinoma is more common in men than in women.

AGE

Hepatocellular carcinoma peaks in the fourth and fifth decade of life. If the younger age group is affected, hepatitis infection with B and C is related to the occurrence of this cancer.

MORTALITY/MORBIDITY

The survival rate of the patient from the time when the cancer is diagnosed is 6 to 8 months. The extent of cirrhosis damaging the liver is directly proportional to the survival period of the patient. Most common factors causing cirrhosis are

1) Alcohol ingestion

2) Hepatitis B or C infection

3) Autoimmune diseases of liver

4) Iron overload in the body (Hemochromatosis)

Liver is a common site for both primary and secondary tumours. 25% of metastatic tumours from various solid organs involve the liver.

The secondary tumours are most commonly adenocarcinomas followed by squamous cell carcinoma and neuroendocrine tumours. Melanomas, lymphomas and rarely sarcomas metastasize to the liver.

Presentation of secondary tumours are usually as multiple umblicated nodules on the surface of the liver rather than solitary lesions. However, histopathological diagnosis is necessary to confirm the lesion.

The image-guiding sampling techniques using fine needle aspiration (FNA) or needle core biopsy(NCB) techniques are the best for diagnosing liver lesion. The key to diagnosis is proper sampling of the lesion.

The important role of a pathologist is to determine the type of the tumour, to differentiate primary from secondary malignancy and in case of secondary tumour, to assess the possible site of origin. After an initial diagnosis of the lesion by routine histopathlogical examination, various immunohistochemical markers may be used for accurate diagnosis of the tumour.

SAMPLING TECHNIQUE AND PREPARATION

Tissue diagnosis is important to start the treatment regime for the patient. The most frequently used modalities to obtain the tissue are Fine Needle Aspiration (FNA) and Needle Core Biopsies (NCB) which may be ultrasound or CT guided. 90% of specimens are obtained in these techniques fetch a good histopathology report. The usage of these both techniques are decided based on the site where the mass is located, the size and the consequences of the complications[5].

Liver happens to be the frequent site of metastatic disease due to its blood supply.

Sampling of the representative area is very important for the final diagnosis. Clinical history of the patient is of great value to the pathologist.

Radiological findings, serum markers and any past history of neoplastic lesion is pertinent[6].

PROCEDURE OF LIVER BIOPSY

The first liver aspirate was performed by the German physician Paul Ehrlich in 1983.

Percutaneous liver biopsy was first reported in the 1920s. The transjugular approach was pioneered by radiologist Charles Dotter in the 1970s.

Before performing liver biopsy, all laboratory investigations including liver function tests, platelet count and coagulation studies are done.

ADEQUACY OF LIVER BIOPSY

According to a study by Atif Zaman et al in 2011, liver biopsy specimen which measures 15 mm in length and 2 mm in diameter and

which is composed of atleast 6 portal tracts is said to be adequate. This accounts to about 1/50,000th of the volume of an adult liver.

Few pathologists consider 4 cm of biopsy sample to be adequate; whereas other few consider 1 cm to be enough to reduce the variations among the observers.

Finally, the exact size of the liver biopsy samples to be received for accurate evaluation still remains inconclusive.

INDICATIONS OF LIVER BIOPSY

1) Evaluation of abnormal laboratory tests
2) Suspected hepatic neoplasms
3) Grading and staging of chronic hepatitis
4) Evidence of granulomatous disease
5) Liver transplantation to evaluate rejection

CONTRAINDICATIONS OF LIVER BIOPSY PROCEDURE

1) Increased prothrombin time
2) Thrombocytopenia
3) Ascites
4) Suspected hemangiomas
5) Suspected echinococcal infection
6) Un co-operative patient

EQUIPMENT

TYPES OF BIOPSY NEEDLES:

CUTTING NEEDLES: Vim-Silverman

SUCTION NEEDLES: Meghini, Klatskin, Jamshidi

PROCEDURE

Patient is placed in a supine position and pillows are removed. He is asked to raise the right arm behind the head, and legs and feet angled to the left. Percussion is done to get the maximum dullness and this site is marked marked with a pen.

Now, local anaesthesia is used and a small incision is made on the skin. Under image guidance (USG or CT), liver biopsy needle is inserted and biopsies are taken.

COMPLICATIONS

1) Pain
2) Hemorrhage
3) Bile peritonitis
4) Bacteremia
5) Sepsis
6) Hemothorax
7) Pneumothorax
8) Accidental puncture of other organs

Many histochemical stains are used to demonstrate the architecture of hepatic parenchyma and for screening of metabolic diseases. These metabolic diseases include haemochromatosis, alpha-1-antitrypsin deficiency and wilson's disease. Depending on the case studied, these stains are used.

The most commonly used stains, apart from haematoxylin and eosin, are reticulin, collagen stain (van Gieson), PAS stain, stain for copper binding protein like orcein and for iron, Perl's stain. Other special stains which can be used are rhodanine, rubeanic acid, acid fast bacilli and amyloid stains.

If no tumour tissue is found in the initial routine haematoxylin and eosin stained section, deeper sections are necessary before giving a negative report. The difficulty arises when there is a well differentiated hepatocellular lesion e.g. well differentiated hepatocellular carcinoma, focal nodular hyperplasia, liver cell adenoma, etc. If additional sections also reveal no lesional tissue, further biopsies and/or investigations are required for diagnosis.

PROCESSING OF THE SPECIMEN

The needle biopsy specimen is gently placed into a fixative as soon as it is received. The tissue must be handled with great care and excessive manipulation of the specimen should be avoided.

Frozen sections may be necessary to demonstrate lipids. Formal saline and buffered formalin are good routine fixatives.

HEPATOCELLULAR CARCINOMA

SMALL HEPATOCELLULAR CARCINOMA

MACROSCOPIC FEATURES

Small hepatocellular carcinomas are tumours less than 2 cm in diameter.

It is difficult to distinguish from a macro-regenerative nodule on gross inspection.They are vaguely nodular with ill defined borders and difficult to distinguish from the surrounding cirrhotic liver.

MICROSCOPIC FEATURES

Small hepatocellular carcinomas are well-differentiated, consisting of thin, irregular trabeculae of crowded hepatocytes.

Mallory hyaline is seen. Reticulin stain shows loss of reticulin fibres. It is distinguished from high-grade dysplastic nodule by increase in nuclear density twice normal but definite nuclear atypia.

ADVANCED HEPATOCELLULAR CARCINOMA

MACROSCOPIC FEATURES

The gross appearance of advanced hepatocellular carcinoma depends on the presence or absence of cirrhosis and the size of the tumour. Tumours arising in a noncirrhotic liver usually are large masses.

They may present with satellite nodules; whereas those associated with cirrhosis present as multiple discrete nodules which cannot be differentiated from cirrhosis.

The liver is large and expanded by one or more tumour nodules. They are green bile-stained and have pale yellow and white areas. They contain foci of necrosis and hemorrhage.

Blood vessel invasion is common if the tumour is large. Tumour nodules at multiple sites are either due to synchronous primaries (multicentric growth) or due to intrahepatic metastasis from the tumour which would have spread through portal vein branches.

MICROSCOPIC FEATURES

Hepatocellular carcinomas which are well differentiated resemble normal liver. Carcinomas of liver were first classified by Edmonson and Steiner who devised a four-tired system based on autopsy studies. This classification was then modified by AFIP and other systems.

Subsequently World Health Organisation devised a system which divides hepatocellular carcinomas into well differentiated, moderately differentiaied and poorly differentiated carcinomas.

Bile located within the neoplastic cells or tubular lumina is characteristic of hepatocellular carcinoma.

Differentiation of the tumour depends on the nuclear grading according to the World Health Organisation classification. Stroma is deficit in hepatocellular carcinoma. Exceptions are fibrolamellar carcinoma and scirrhous carcinoma.

Tumours with high nuclear grading are associated with poor survival rate and show positivity in positron emission tomography (PET) imaging.

Edmonson and Steiner devised a four-tired system based on autopsy data. This was subsequently modified in a large series reported from the AFIP and other similar systems have been proposed. The World Health Organization Classification divides tumours into well, moderately and poorly differentiated grades. Most tumours are moderately differentiated and more than one histological grade is often present within a tumour.

Although tumour grade has not universally been shown to have a significant impact on outcome, higher nuclear grade has been reported to predict poorer survival in hepatocellular carcinomas resected with curative intent and higher tumour grade corresponds to positivity on positron emission tomography (PET) imaging.

HISTOLOGICAL PATTERNS

Multiple histological patterns are observed. These patterns are not important to assess the prognosis except in case of fibrolamellar variant; but recognizing these different patterns are useful so that they are not misdiagnosed as metastatic carcinomatous deposits involving the liver. These patterns are as follows:

TRABECULAR

This is a commonly found pattern which resembles normal hepatic architecture.The tumour cells grow in cords or plates separated by vascular channels lined by endothelial cells and kupffer cells, with little intervening stroma. The trabeculae vary in thickness, from only a few cells thick to broad structures 20 or so thick. The reticulin framework is reduced or absent.

COMPACT

Its occurrence varies from 5 to 15%. Confluent growth results in a solid growth pattern, with inconspicuous or obliterated sinusoids.

PSEUDOGLANDULAR (ACINAR)

This pattern is important to recognize because it is often mistaken for metastatic adenocarcinoma, cholangiocarcinoma or hepatocellular carcinoma combined with cholangiocarcinoma. The tumour is composed of spaces which are not true glands; they represent dilated bile canaliculi lined by a single layer of tumour cells. These pseudoglands appear to be freely floating and no fibrous stroma is seen in the background. This feature helps to distinguish from adenocarcinomatous deposits in the liver.

These spaces between the tumour cells sometimes have acellular eosinophilic material which may show positivity for PAS but it is negative for mucicarmine and alcian blue.

The following categories are recognized cytological variants of hepatocellular carcinoma:

PLEOMORPHIC (GIANT CELL)

This variant contains tumour cells which show marked variation in size and shape. Bizzare cells are also noted. The trabecular pattern often

seen in well differentaiated hepatocellular carcinoma is often lacking in this variant.

There is loss of cohesion of tumour cells which indicates that this is a high grade variant of hepatocellular carcinoma. In these cases extensive sectioning is mandatory to find any focus of typical hepatocellular carcinoma.

CLEAR CELL

This type of hepatocellular carcinoma is called so because it contains tumour cells with abundant clear cytoplasm due to glycogen and / or fat. This variant has to be differentiated from other neoplasms with clear cytoplasm like metastatic renal, adrenal and ovarian carcinomas. Additional sections have to be taken to find evidence of typical hepatocellular carcinoma. In these instances serum AFP level, presence of chronic liver disease and the absence of extra-hepatic mass may lead one to correct diagnosis of hepatic primary neoplasm.

SARCOMATOID (SPINDLE CELL, PEUDOSARCOMATOUS)

This accounts for about 4% of hepatocellular carcinomas. Areas with spindle shaped cells with nuclear atypia constitute the sarcomatoid foci. There may be both carcinomatoid and sarcomatoid foci in the same tumour. Areas resembling fibrosarcoma or malignant fibrous

histiocytoma may be seen. Hence, extensive sampling is needed to demonstrate the primary nature of the tumour.

FIBROLAMELLAR HEPATOCELLULAR CARCINOMA

This variant justifies its separation from other types of hepatocellular carcinoma because of is distinct clinical and pathological features and natural history. The patients are younger than those with other types of hepatocellular carcinoma. Its incidence is equal in men and women.

The fibrolamellar variant is not associated with chronic liver disease, cirrhosis or any other known risk factor.

Grossly, the tumours are hard unlike other hepatocellular carcinomas, due to the presence of fibrous stroma. Central scarring may be seen in the tumour.

Microscopically, the tumour is composed of distinctive cytological features with a fibrous stroma. The stroma is thick and contains hyalinised collagen bundles. These bundles of collagen are arranged in parallel lamellae of varying thickness, so the name fibrolamellar.

The cells are large and polyhedral. They have a dense eosinophilic cytoplasm due to abundant mitochondria. Approximately half of the

tumours have ground glass cytoplasmic inclusion bodies called ‘pale bodies’. Bile may be seen within the tumour cells. Mitotic activity is low.

These tumours are slow growing and are often surgically resectable; hence, they carry a better prognosis compared to other types of hepatocellular carcinoma.

ROLE OF RADIOGRAPHY IN THE DIAGNOSIS OF HEPATIC MALIGNANCIES

Radiography i.e. CT or MRI scan is usually performed in case of mass lesions of liver. CT scan shows increase in vascularity, calcifications and fat within the lesion.

CT and MRI imaging can assess

1) whether the tumour is single or multiple
2) size of the tumour
3) whether the tumour is well defined or ill defined.

The treatment of hepatocellular carcinoma depends on the

1) the size of the tumour
2) stage at which the tumour is diagnosed
3) vascular and capsular invasion

4) presence or absence of metastatic disease

5) whether the tumour is hypervascular or hypovascular.

Radiographically, most metastatic adenocarcinomas of liver show decrease in vasularity.

PROGNOSIS OF HEPATOCELLULAR CARCINOMA

The survival rate of the patients with hepatocellular carcinoma is generally worse. Most patients die within a year. The reasons for such poor prognosis are the large tumours which present late and insufficient medical facilities for proper diagnosis and treatment especially in the developing countries.

The prognosis of patients with metastatic disease is generally poor; and the treatment options include reception, cryoablative therapy and ethanol injection. All these modalities give a palliative cure in the management of those patients with metastatic disease involving the liver.

PRECURSORS OF HEPATOCELLULAR CARCINOMA

These include hepatocellular changes and nodular lesions. These lesions are important to identify because they need patient surveillance and if necessary surgical treatment.

They are as follows:

Liver cell dysplasia:

a) Small cell type

b) Large cell type

Large-cell liver cell dysplasia (large cell change)

Large cell type of liver cell dysplasia is common. It shows nuclear enlargement, pleomorphism, multinucleation and multiple nucleoli. It is seen in hepatitis B virus infection, cirrhosis and hepatocellular carcinoma. They also show aneuploidy. It is strongly due to cholestasis or normal cell-polypoidisation. The large cell dysplasia is an important independent risk factor for hepatocellular carcinoma.

Small-cell liver cell dysplasia (small cell change)

This shows enlarged and hyperchromatic nucei arranged in clusters. There is high cell proliferation rate and may lead to hepatocellular carcinoma.

Other cellular changes indicated in pre-malignant conditions include intracytoplasmic Mallory bodies, areas of regeneration which show glycogenesis and oncocytic change in hepatocytes or bulging nodularity, siderotic macroregenerative nodules which show iron negative foci and livers of patients with hereditary haemochromatosis which have iron free foci.

The dysplasia may be further categorised into

i. Low grade dysplasia and
ii. High grade dysplasia

When clusters of large-cell or small-cell dysplastic hepatocytes less than 1 mm are seen, they are called as dysplastic foci.

MACROREGENERATIVE NODULE

This refers to a regenerative nodule larger than 8 mm; it develops more commonly in cirrhotic liver. The livers with macroregenerative nodules may also have associated hepatocellular carcinoma.

The macroregenerative nodule under microscopy shows hyperplastic hepatocytes in plates of two or three cells thick. There is no cellular atypia seen. The portal tracts and fibrous septa containing bile ducts, hepatic arteries and portal vein branches are seen within the

nodule. Fatty change, biliary plugging and Mallory bodies are often noted.

DYSPLASTIC NODULE

The dysplastic nodules do not show features of macroregenerative nodule both cytologically and architecturally. They show changes of large and small-cell liver cell dysplasia. The cells are large and small with increased cellularity, loss of cohesiveness. Reticulin is also absent within the nodule.

The macroregenerative nodules and the dysplastic nodules should be differentiated from hepatocellular carcinoma. Features for carcinoma are increased nuclear cytoplasmic ratio, dysplastic cells, fatty change increased mitosis, broad trabeculae, infiltrative margins and absence of reticulin.

BENIGN AND BORDERLINE HEPATOCELLULAR TUMOURS

1) Hepatocellular adenoma
2) Focal nodular hyperplasia and
3) Nodular regenerative hyperplasia

are the three notable lesions of liver which are entirely benign. These lesions are to be differentiated from the lesions which are considered as the potential precursors of hepatocellular carcinoma.

The above three proliferations originate in the normal liver; whereas the precursors more commonly arise in a cirrhotic liver.

HEPATOCELLULAR ADENOMA

It is a benign tumour of liver which arises in a non-cirrhotic background. It is more common in middle aged women who take oral contraceptive pills and in men who take anabolic steroids.

Clinically it can be differentiated from hepatocellular carcinoma by the serum level of Alpha Feto-protein which is within normal limits in case of hepatocellular adenoma.

GROSS

The tumour is usually solitary and it is well delineated from the surrounding normal liver. The colour of the tumour is generally grey yellow. But areas of necrosis and hemorrhage may be seen within the tumour.

MICROSCOPY

The hepatocellular adenoma may be confused with normal liver when viewed with a low power. But on careful observation it may be noted that unlike normal hepatic parenchyma, no portal tracts or biliary ducts are made out within the tumour. Instead arteries and veins which

are randomly distributed are seen. This is an important feature of this lesion.

The individual cells may show fatty change. Focal nuclear atypia is sometimes seen but mitosis is absent.

The tumour in most cases is entirely benign. But it is observed that a category of the tumour caused by mutation in beta-catenin gene has a propensity to develop hepatocellular carcinoma.

The differential diagnosis includes well-differentiated hepatocellular carcinoma. The differentiating features include significant nuclear pleomorphism and prominent nucleolus. Another important clue to diagnosis is that reticulin is lost in hepatocellular carcinoma and is retained in hepatocellular adenoma.

The prognosis of hepatocellular adenoma is good, many cases having a tendency to regress when the hormonal drugs are withdrawn.

Surgical excision is generally curative. But surveillance is needed if hormones are not known to be the risk factors, the tumour shows nuclear pleomorphism or when the tumour is not resected completely.

FOCAL NODULAR HYPERPLASIA

This lesion is also a benign proliferation of hepatocytes. Unlike the hepatocellular adenoma which is more common in women, the incidence of focal nodular hyperplasia is equal in both sexes.

The exact etiology of focal nodular hyperplasia is not clear; but it is observed that this is a localized proliferation of benign hepatocytes which is seen around an anomalous vessel. Focal nodular hyperplasia like lesions can develop in a liver with cirrhosis and so this lesion is important to be recognized because of its prognostic significance.

GROSS

The lesion usually presents as a single mass beneath the capsule. Cut surface is usually grey white to tan in colour. A central radiating scar is seen in many cases especially if the mass is less than 10 mm in size.

HISTOLOGY

Focal nodular hyperplasia, as the name indicates, is composed of non-neoplastic hepatocytes arranged in nodules separated by thick fibrous septa. Bile ducts, few branches of arteries and inflammatory infiltrate are seen within the septa. Similar to hepatocellular adenoma, portal tracts and bile ducts are absent within this nodule.

These lesions have entirely benign course and need no resection unless symptomatic.

NODULAR REGENERATIVE HYPERPLASIA

This is less common lesion when compared to focal nodular hyperplasia and hepatocellular adenoma. It is important to diagnose the lesion because of its close resemblance to cirrhosis grossly; and the prognosis varies between the two conditions. Nodular regenerative hyperplasia involves the liver in a diffuse manner.

GROSS

The cut surface of the liver with nodular regenerative hyperplasia shows multiple tan coloured nodules which diffusely involve the liver which closely resembles cirrhosis.

MICROSCOPY

The characteristic histopathological picture is hyperplastic nodules of benign hepatocytes separated by atrophic hepatocytes without significant fibrosis; this differentiates it from cirrhosis. Congestion of sinusoids and compression of central veins are seen. The hepatocytes which are found resemble normal hepatocytes and those seen between the nodules are small and atrophic.

This pattern of alternative regenerating and the atrophic hepatocytes is demonstrated by reticulin stain.

Differential diagnosis includes cirrhosis of liver. This difficulty can be overcome with the help of special stains like trichrome and reticulin stains.

RETICULIN

Reticulin is used to describe a type of fibre which is seen in the connective tissue. It is made of type 3 collagen[11]. Reticulin fibres cross link to form a fine meshwork (reticulin). This reticulin network acts as a supporting mesh in various soft tissues like liver, bone marrow, and the tissues and organs of lymphatic system.

The Bielschowkys' technique of reticulin staining by silver impregnation was found by Maresch in 1905.

Since the 1920s, the studies on "reticulin" distribution were based entirely on silver impregnation technique. Gomori's and Wilder's methods to stain reticulin fibres in the cells of various organs revealed different staining patterns.

STRUCTURE

The fibres of reticulin are made up of various types of type 3 collagen which are very thin and delicate strands. Thus, the collagen

strands yield an orderly network for a good support to the tissues. These strands of collagen are seen connected to carbohydrate moiety. Hence, they stain both reticulin and carbohydrate stains but cannot be seen in routine hematoxylin and eosin stained sections. Since they have affinity for silver salts, these fibres are termed argyrophilic.

PRINCIPLES OF RETICULIN STAINING

Stains used for reticulin, as already mentioned are silver stains which are depend on the argyrophilia of the reticular fibres. There are two commonly used reticulin stains; these are the Gomori's stain and the Gordon and Sweet's stain.

UTILITY OF RETICULIN

The fine network of reticular fibres are especially seen in the hepatic parenchyma at the site of trabeculae. This network of reticulin gives good connective tissue support to the liver. The basis of this information gives valuable knowledge regarding the morphology of hepatic parenchyma.

If necrosis occurs in hepatocellular parenchyma due to some damage, the reticulin fibres surrounding the hepatocytes are collapsed leaving a space behind. So, when reticulin fibres are seen crowded, it indicates focal hepatocyte loss.

If regeneration of cells of liver hepatocytes occurs, these fibres of reticulin reveal increase in thickness of liver cell plates of more than one-cell.

In a study by Norredam K in 1979, among 7763 autopsies performed, there were 309 cases of cirrhosis of the liver and 52 cases of carcinomas of liver. Of the latter, 45 were hepatocellular carcinomas, 4 combined hepatocellular carcinoma and cholangiocarcinoma and 3 cholangiocarcinoma. The reticulin stain was found very valuable in hepatocellular carcinoma both for descriptive and diagnostic purposes.

According to a study by Bergman and Graeme in 1997[67], the stain pattern was analyzed using semi-quantitative system: normal, variable, decreased and absent based on whether the cells of hepatic parenchyma are than or less than three-cells thick.

About 90% of cases of benign hepatic lesions showed normal network of reticular fibres i.e. took the stain along the trabeculae which are not more than three cell thick. There seemed to be great reduction in a specimen of fatty liver and it was totally absent in a biopsy specimen of cirrhotic liver. On the other hand, every case of hepatocellular carcinoma showed a totally absent , reduced or variable pattern of reticulin staining, revealing an increase in thickness.

METASTATIC CARCINOMATOUS DEPOSITS

ADENOCARCINOMAS

Adenocarcinomas are the most common carcinomas to metastasize to the liver. In order of frequency are pulmonary, colonic, pancreatic and gastric malignancies accounting for approximately 25%, 16%, 11% and 6% of the cases respectively. Other carcinomas like ovarian, uterine, prostatic and biliary carcinomas represent 4% each.

Adenocarcinomas represent those tumours which arise in a glandular tissue. The most common morphology is glandular pattern lined by tall columnar cells, which is similar to the glands seen in the native organ.

The adenocarcinoma shows focal mucin production, and forms signet ring cells and acinar pattern. Mucin can be demonstrated with a histochemical stain like mucicarmine.

The exact site of primary is difficult to assess with the help of morphological characteristics alone; but still some primary sites can be suspected which helps in the diagnosis in needle core biopsies. The tumours which fall in this category are those from colon, breast, pancreaticobiliary tract,etc...

Colorectal carcinoma shows a central dirty necrosis in the glands. Ductal adenocarcinoma of breast reveals a monomorphic population. Lobular carcinoma is seen with cells infiltrating in single file pattern. The cells with a targetoid cytoplasmic lumen are helpful in the diagnosis of mammary carcinomas.

Pancreaticobiliary carcinomas do not have a specific pattern but may be suspected when they show abundant cytoplasmic mucin or clear nuclei. However, a good clinical history is needed to differentiate primary cholangiocarcinoma from metastatic pancreaticobiliary carcinoma.

The morphological characteristics of other various carcinomas like pulmonary, uterine, esophageal carcinomas and in the stomach donot have their specific findings for their diagnosis in the biopsies.

SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma is a rare metastatic tumour to the liver. The carcinomas which should be kept on mind when dealing with the origin are pulmonary and esophageal tumours, and carcinomas from head and neck, gonads and anorectum. The individual cells reveal keratinisation as an evidence for the diagnosis; however this is not always mandatory.

Immunohistochemistry is also not useful. Only detailed history regarding the patient's clinical status may be helpful in identifying the primary site.

NEUROENDOCRINE CARCINOMA

Neuroendocrine carcinomas have various degrees of differentiation. The cells of benign tumours like carcinoids appear monotonous showing minimal mitosis. The distinct salt and pepper pattern of chromatin is seen in these cells.

Carcinoids arise anywhere in the gastrointestinal tract. Highly malignant tumours like small cell carcinoma arising in the lung are common. They reveal moulding of nuclei high mitotic rate, areas of necrosis and crush artifacts as seen in the primary lung carcinoma.

Immunohistochemical studies are useful in confirming the diagnosis of neuroendocrine tumours. The markers used in the diagnosis are synaptophysin, chromogranin and neuron specific enolase.

MUCINOUS CARCINOMA

This is a specific category of adenocarcinoma. It can be diagnosed only when the tumour contains more than 50% of extracellular mucin. These tumours are commonly from large intestine, breast, ovary, pancreas but can occur anywhere along the gastrointestinal tract. It can also be a mucinous broncheolo-alveolar carcinoma

There are two histological patterns seen in mucinous carcinomas:

1) Tumour cells floating in pools of mucin
2) The individual tumour cells are tall columnar with intracytoplasmic mucin.

RENAL CELL CARCINOMA

The clear cell carcinoma of kidney infrequently metastasizes to the liver. Here, the cells are clear with bland nuclei and abundant blood vessels in the stroma. The other types are papillary renal cell carcinoma and chromophobe renal cell carcinoma.

MELANOMA

Melanomas are called as the great mimics in pathology. The morphology of the tumour cells in melanoma is so variable that any carcinoma can under into the differential diagnosis. Moreover, many patients present with metastasis long after the primary has been diagnosed.

But, liver is an infrequent site for metastasis of melanoma, accounting for appropriately 2.2%. Cells can be identified by intranuclear inclusions and prominent nucleoli.

LYMPHOMA

The appearance of lymphoma is based on the type of lymphoma. Diffuse large B cell lymphomas are the first among lymphomas to

metastasise to the liver; they are easily diagnosed by their large size and nuclear and chromatin characteristics.

Follicular lymphomas, mucosa associated lymphoid tissue (MALT) lymphomas and small lymphocytic lymphomas are the other types of lymphomas; but there is difficulty in diagnosing these tumours based on morphology alone; and the clinician should be asked regarding any history of lymphoma in the patient.

SARCOMA

The most common spindle cell tumours to secondarily involve the liver are gastrointestinal stromal tumours and leiomyosarcomas. The gastrointestinal stromal tumours show spindle cells with moderate amount of eosinophilic cytoplasm and oval nucleus; nuclear pleomorphism may be noted. The tumour cells show positivity for c-kit in the cytoplasm.

Leiomyosarcomas reveal tumour cells which are highly pleomorphic when compared to gastrointestinal stromal tumours. In difficult cases immunohistochemistry with desmin and smooth muscle actin may be useful.

MISCELLANEOUS TUMOURS

Urothelial carcinomas can also metastasize to the liver. They show cells that are seen in sheets and the cytoplasm is eosinophilic to amphophilic with elongated grooved nucleus.

ROLE OF IMMUNOHISTOCHEMISTRY IN HEPATIC NEOPLASMS:

HEPATOCELLULAR CARCINOMA VERSUS METASTATIC NEOPLASMS:

Differentiating hepatocellular carcinoma from other neoplasms of the liver can be difficult and is often challenging in core and fine needle aspiration biopsies.

Several immunohistochemical markers are available which aid in this differential diagnosis.

DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

The most commonly encountered differential diagnostic challenge in the liver is hepatocellular carcinoma versus intrahepatic cholangiocarcinoma and metastatic malignancies like neuroendocrine tumours, renal cell carcinoma, adrenocortical carcinoma, melanoma and epithelioid angiomyolipoma.

Lau SK and Prakash et al in 2002[28] evaluated Hep Par1 along with other IHC markers to differentiate hepatocellular carcinoma, cholangiocarcinoma and metastatic adenocarcinoma. 42 cases of HCC, 9 cases cholangiocarcinoma and 56 cases of metastatic adenocarcinoma (24 colon, 15 pancreas, 8 ovary, 5 breast and 4 stomach) were studied.

Hep Par1 was found to be a good marker, being both sensitive and specific for HCC, accounting for most of the cases, approximately 89% showing positivity; also only about 13% of metastatic carcinoma cases and none of the cholangiocarcinoma cases were positive for this marker. The markers p-CEA, CD10 and villin were found to be more specific for HCC since they were negative in all other malignancies.

Absence of monoclonal carcino-embryonic antigen and MOC-31 staining was observed characteristic for hepatocellular carcinoma. Cytokeratin 7 and Cytokeratin 20 were found to be useful in the diagnosis, especially in cases of metastatic adenocarcinomas arising in the colon.

COMMONLY USED MARKERS:

HEP-PAR 1: (Also known as hepatocyte antigen):

Hep-Par 1 is a monoclonal antibody that is considered as the most sensitive and specific immunohistochemical marker for hepatocellular carcinoma. It was first developed from failed allograft liver. Hep Par 1 gives a diffuse cytoplasmic granular staining pattern in both normal and neoplastic hepatocytes.

But Hep Par 1 is not helpful in differentiating benign from malignant hepatocellular lesions because of its positive staining pattern in normal liver and in adenomas.

ADVANTAGES

a) It is highly sensitive and specific (both >80%).

b) It may be negative in metastatic adenocarcinomas from biliary tree, pancreas, colorectum, breast, urinary bladder and prostate.

c) The tumours which may be confused with hepatocellular carcinoma by light microscopic, such as neuroendocrine neoplasms, renal cell carcinoma, malignant melanoma and epithelioid angiomyolipoma, are usually negative, of only focally positive for Hep Par 1.

PITFALLS IN DIAGNOSIS

a) The sensitivity of Hep Par 1 is low (50% or less) in poorly differentiated carcinomas.

b) In about 20% of hepatocellular carcinomas, Hep Par 1 yields only patchy staining and so needle biopsies may be negative for the marker.

c) Certain carcinomas have hepatoid morphology (Eg. gastrointestinal tract and pancreas); and these are positive for Hep Par 1.

ALPHA FETOPROTEIN (AFP)

AFP was first identified in 1956 on paper electrophoregrams by Bregstrand and Czar. After a few years, it was concluded that Alpha Feto Protein is a useful marker in differentiating hepatocellular carcinoma from metastatic deposits in liver.

Other than hepatocellular carcinoma, AFP was also found to be associated with teratoblastoma of the testis and ovarian germ cell tumours.

AFP is an oncofetal protein produced by the liver and visceral endoderm of yolk sac. It is specifically expressed in tumour cells of hepatocellular differentiation, provided germ cell tumours are excluded.

But AFP has a patchy staining and low sensitivity of 30 to 50%. Because of low sensitivity and availability of better antibodies, AFP is not considered as a good option for diagnosis.

Porcell et al in 2000[41] studied 57 previously characterized hepatic neoplasms, 13 cases were hepatocellular carcinomas. Alpha Fetoprotein was detected in 4 of these 13 cases (30%).

In a study by Lau SK Prakash S et al in 2002[28], 42 cases of hepatocellular carcinomas were studied using a panel of markers including Alpha Fetoprotein, Hep Par 1, p-CEA, CD10 and villin. They found that Alpha Fetoprotein was observed in one third of the cases.

GLYPICAN -3 (GPC-3)

Glypican-3 is a membrane anchored heparan sulphate proteoglycan and it is expressed in fetal liver and placenta normally but not in adults. Studies have shown that GPC-3 is positive in 64% to 90% of hepatocellular carcinomas and negative in normal liver or benign hepatic lesions like adenomas.

ADVANTAGES

a) It is highly sensitive (about 80%).

b) More useful and sensitive than Hep Par 1 for poorly differentiated hepatocellular carcinoma.

c) It can be used to distinguish benign from malignant hepatocellular lesions since it is negative in normal liver and hepatic adenomas.

PITFALLS IN DIAGNOSIS

a) It's a relatively newer antibody and more extensive studies in other or needed to confirm its reported high sensitivity.

b) It may be negative in well differentiated hepatocellular carcinomas.

c) GPC-3 is not very specific because it gives positive staining pattern in melanoma and non-seminomatous germ cell tumours such as yolk sac tumour and choriocarcinoma.

POLYCLONAL CARCINOEMBRYONIC ANTIGEN (CEA)

In a study conducted by Mack and Zarbo RJ et al in 1993[30], who studied 56 hepatocellular carcinomas, 71% of hepatocellular carcinomas revealed a bile canalicular staining pattern with pCEA.

CEA is a glycoprotein which is present in fetal epithelial cells and in normal adult cells. Polyclonal carcinoembryonic antigen (p-CEA) yields diffuse cytoplasmic staining many cases of adenocarcinoma.

In hepatocellular carcinoma, it gives a characteristic canalicular pattern which is seen in 60% to 90% of cases. This staining is due to cross reaction with biliary glycoprotein.

This characteristic canalicular pattern of hepatocellular carcinoma is absent in adenocarcinoma. Monoclonal CEA also shows positivity in adenocarcinomas, but its sensitivity is low (around 80%). Hepatocellular carcinoma is negative for monoclonal CEA.

ADVANTAGES

a) The characteristic canalicular staining pattern is specific for hepatocellular carcinoma.

b) p-CEA has sensitivity of more than 80% in well and moderately differentiated carcinoma.

PITFALLS IN DIAGNOSIS

a) Interpretation of cytoplasmic and canalicular patterns of staining is difficult in some cases.

b) Around 50% of hepatocellular carcinomas can show diffuse cytoplasmic staining for p-CEA, in addition to canalicular pattern.

c) Similar to Hep Par 1, its sensitivity is low (25 to 50%) in poorly differentiated hepatocellular carcinoma.

MOC – 31

MOC-31 is an immunohistochemical marker which was found to be helpful to separate the cases of metastatic adenocarcinomatous deposits and mesothelioma. It is noted in 80 to 100% cases of cholangiocarcinoma and metastatic adenocarcinoma from a variety of sites, such as colorectum, pancreas, stomach, lung, breast and ovary.

CD10 AND VILLIN

CD10 and villin, like p-CEA show canalicular pattern in hepatocellular carcinoma. They are not as good as p-CEA, since they are less sensitive. (50% for CD10 and 20% for villin).

TTF-1

TTF-1 is normally expressed in the follicular epithelial cells of thyroid and also in the lung; and so, in tumours that arise from them. Cytoplasmic TTF-1 staining is noted in about 10% of hepatocellular carcinomas.

CYTOKERATINS(CK)

CK8 and CK18 are expressed in normal and neoplastic hepatocytes; whereas CK7, CK19 and CK20 are negative in these sites. CK cocktail AE1/AE3 shows only patchy staining except in cases of poorly differentiated hepatocellular carcinomas which may show clusters of positive cells.

CK7 and CK20 are negative in most cases of hepatocellular carcinomas.

Nearly 75% of hepatocellular carcinomas are CK7 and CK20 negative, 20% are CK 7 positive and CK20 negative and 5% are CK7 positive and CK20 positive. Like AE1/AE3, CK7 tends to be stronger in poorly differentiated hepatocellular carcinoma.

Hence, the commonly used keratin antibodies (AE1/AE3, CAM5.2, CK7, CK20) can be expressed in both hepatocellular carcinoma and adenocarcinoma, limiting their value in this differential diagnosis.

CK7 and CK20 are also useful in assessing the possible primary site once the diagnosis of adenocarcinoma is made. CK19 shows positivity in 85% to 100% of cholangiocarcinoma and it is negative in most hepatocellular carcinomas.

CD34

CD34 is normally not expressed in the sinusoids in the normal liver but the sinusoid-like vasculature in hepatocellular carcinoma often shows strong expression of CD34, which is due to the capillarization of sinusoids leading to a change in the phenotype of endothelial cells.

This sinusoidal pattern of CD34 expression is specific for hepatocellular carcinoma because it is not seen in adenocarcinoma. But it has a low sensitivity of 20% to 40%.

It is not useful in differentiating benign from malignant hepatocellular lesions because it can also be seen in focal nodular hyperplasia and hepatic adenomas. But CD34 may be useful to differentiate well differentiated hepatocellular carcinoma from a normal or cirrhotic liver in case of small biopsy specimens.

Albumin in situ hybridization is specific and sensitive (>90%) for hepatocellular differentiation. Combination of albumin in situ hybridization and Hep Par1 can yield 100% sensitivity for diagnosis of

hepatocellular carcinoma. However, the use of this test is limited by its restricted availability[26].

Materials and Methods

MATERIALS AND METHODS

Cases diagnosed as hepatic malignancy on liver biopsy specimens received in the Department of Pathology, Coimbatore Medical College Hospital during a period from July 2011 to July 2012 were taken.

INCLUSION CRITERIA

1) Liver biopsy specimens reported as dysplastic and neoplastic lesions of liver.

2) Patient age more than 12 years.

EXCLUSION CRITERIA

1) Liver biopsy specimens other than dysplastic and neoplastic lesions of liver.

2) Patient age less than 12 years.

Sections were cut at 4 microns thickness. Coated slides were used and the slides kept in incubation at 58 degrees overnight. The initial sections were stained with hematoxylin and eosin stain.

The unstained slides were used for running reticulin stain by Gomori's method and immunohistochemistry by a two-step indirect technique.

METHOD OF HEMATOXYLIN AND EOSIN STAINING

Reagents used :

1. Erhlich's Hematoxylin solution
2. Eosin Y solution 1 %
3. Acid Alcohol solution 1%

Procedure :

1. Deparrafinize the sections
2. Immerse the sections in xylene for 30 minutes
3. Then place in isopropyl alcohol for 15 minutes
4. Wash in tap water
5. Stain the sections with Erhlich's Hematoxylin for 10-15 minutes
6. Wash in tap water
7. Differentiate in 1% acid alcohol solution – 2 to 3 dips
8. Blueing for 10 minutes.
9. Counter stain with 1% eosin solution - 2 to 3 dips
10. Wash in tap water
11. Air dry the sections
12. Xylene – Mount

These hematoxylin and eosin stained sections are then observed and a diagnosis is obtained based on histomorphological features.

METHOD OF RETICULIN STAIN

Gomori's method

PRINCIPLE

Reticulin staining is based on the high content of hexose sugars in reticulin.

Reticulin fibres have little natural affinity for silver solutions and hence they are pretreated to produce sensitized sites where silver will deposit.

REAGENTS USED

1) Ammoniacal silver solution
2) 0.5% KMnO4
3) 2% Potassium metabisulfite
4) 2% Ferric Ammonium Sulfate
5) 20% Formalin
6) 0.25 Gold chloride
7) 2% Sodium thiosulphate

PREPARATION OF AMMONIACAL SILVER SOLUTION

10% Silver nitrate : Add 1gm of silver nitrate to 10 ml of distilled water.

10% Aqueous solution of KOH : Add 1 gm of KOH to 10 ml of distilled water.

Mix 10 ml of 10% silver nitrate solution with 2.5 ml of 10% aqueous solution of KOH.

Then, add 28% ammonia drop by drop until the precipitate is completely dissolved. Add an equal amount of distilled water.

PROCEDURE

1) Deparrafinze tissue sections in xylene for 30 minutes.
2) Wash in absolute alcohol for 10 minutes with 2 changes.
3) Place in tap water for 10 minutes and subsequently in distilled water for 5 minutes.
4) Oxidise the sections in KMnO4 solution.
5) Wash in tap water and differentiate in potassium metabisulfite.
6) Sensitize in ferric ammonium sulphate for 1 minute.
7) Wash in tap water for 2 minutes and distilled water for 5 minutes.
8) Impregnate with silver solution.

9) Rinse in distilled water and then reduce in formalin solution.
10) Wash in tap water and tone in gold chloride for 10 minutes.
11) Then reduce in potassium metabisulfite and fix in sodium thiosulfate.
12) Wash in tap water, alcohol and xylene.
13) Air dry and mount with DPX.

RESULT

Reticulin fibres are stained black in a colourless background.

METHOD OF IMMUNOHISTOCHEMISTRY

METHOD

Two-step indirect technique.

PRINCIPLES OF THE PROCEDURE

This technique is based on the detection of antigens in the cells and tissues with the help of a two-step process:

1. Specific epitopes used to bind the primary antibody followed by,
2. Colorimetric reaction used to detect this binding.

The tissue sections which are paraffin embedded are taken and antigen retrieval is done. This is performed using a microwave. The tissue sections are placed in appropriate buffer solutions and then subjected to heating in a microwave. This helps to retrieve the antigenicity of the cells.

Now the sections are treated with power block to block the non-specific interactions between the proteins.

REAGENTS USED

1) Peroxide Block: 3%hydrogen peroxide in water.
2) Power Block Reagent: A highly effective universal protein blocking reagent. Contains casein and propriety additives in PBS with 15mM sodium azide.
3) Chromogen: DAB-3,3'-diaminobenzidine.
4) Liquid DAB Substrate: Comprises Tris buffer containing the peroxide and stabilizers.
5) Super Enhancer Reagent.
6) Poly-HRP Reagent.
7) Counter stain: Mayer's Hematoxylin.
8) Buffer solutions:

TRIS BUFFER: (ph -7.6)

TRIS Buffer salt: 0.605 gm

Sodium chloride: 8 gm

Distilled water: 1000 ml

1N Hydrochloric acid: 3 ml

CITRATE BUFFER: (ph-6.0)

Trisodium citrate: 2.94 gm

Distilled water: 1000 ml

1 N Hydrochloric acid: 5 ml

TRIS EDTA: (ph-9.0)

TRIS Buffer salt: 6.05 gm

Disodium EDTA: 0.744 gm

Distilled water: 1000 ml

PROCEDURE

1) Deparaffinise the sections in xylene for 30 minutes.
2) Wash in absolute alcohol for 5 minutes with 2 changes.
3) Wash the slides in tap water for 10 minutes.
4) Rinse in distilled water for 5 minutes.
5) Antigen retrieval is done by placing the slides with appropriate buffer solution in microwave: Medium-10 minutes: High-10 minutes.
6) Cool to room temperature and rinsed in distilled water.
7) Wash in TBS buffer for 5 minutes with 2 changes.
8) Treat with Peroxide Block for 10 minutes.
9) Wash in TBS buffer for 5 minutes with 2 changes.

10) Treat with Power Block for 10 minutes.

11) Drain the slides and cover with primary antibody (supplied from DAKOCYTOMATION) for 2 hours.

12) Wash in TBS buffer for 5 minutes with 2 changes.

13) Cover the slides with Super Enhancer for 30 minutes.

14) Wash in TBS buffer for 5 minutes with 2 changes.

15) Apply Poly HRP reagent and leave for 30 minutes.

16) Wash in TBS buffer for 5 minutes with 2 changes.

17) Treat with DAB Chromogen with Substrate buffer for 5 to 8 minutes.

18) Wash in TBS for 5 minutes with 2 changes.

19) Wash the slides in tap water for 5 minutes.

20) Counterstain with Mayer's Hematoxylin for 1 minute.

21) Wash in tap water for 5 minutes.

22) Air dry and mount with DPX.

Tumour cells are scored positive if there is golden brown cytoplasmic staining in the neoplastic cells.

Observation

OBSERVATION

A total of 34 liver biopsies were reported in the Department of Pathology, Coimbatore Medical College Hospital over a period from July 2011 to September 2012. Out of these, 26 cases were reported as hepatic neoplasms. Among these hepatic neoplasms, 13 cases were reported as hepatocellular carcinomas and 13 cases as metastatic carcinomatous deposits in the liver. Thus, the incidence of hepatic neoplasms among the liver biopsy specimens received was 76.4%.

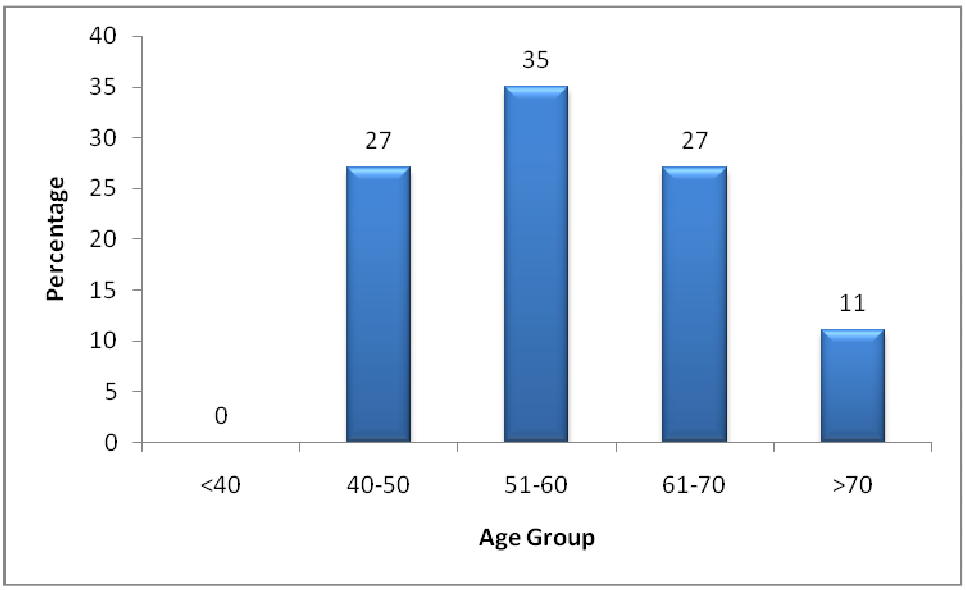
TABLE-1

INCIDENCE OF HEPATIC MALIGNANCY IN DIFFERENT AGE GROUPS

S.No.	AGE GROUP (Yrs)	No. OF CASES	PERCENTAGE (%)
1.	<40	0	0
2.	40-50	7	27
3.	51-60	9	35
4.	61-70	7	27
5.	>70	3	11
	TOTAL	**26**	**100**

CHART-1

INCIDENCE OF HEPATIC MALIGNANCY IN DIFFERENT AGE GROUPS



In the above frequency table, it is observed that the incidence of hepatic malignancies was high (35%) in the age group of 51 to 60 yrs, followed by the age group of 41-50 yrs (27%), 61-70 yrs (27%) and >70 yrs (11%).

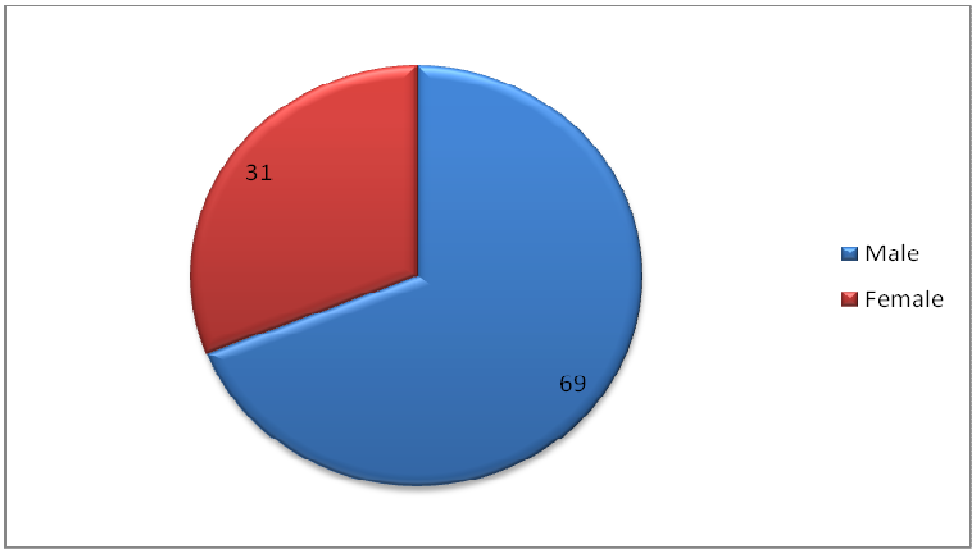
TABLE-2

INCIDENCE OF HEPATIC MALIGNANCY IN DIFFERENT SEX GROUPS

S.No.	SEX	No. OF CASES	PERCENTAGE (%)
1.	Male	18	69
2.	Female	8	31
	TOTAL	**26**	**100**

CHART- 2

INCIDENCE OF HEPATIC MALIGNANCY IN DIFFERENT SEX GROUPS



It is observed from the above table that the incidence of hepatic neoplasms was comparatively high in males (69%) than in females (31%).

In the present study, among 13 cases reported as hepatocellular carcinomas in Hematoxylin and Eosin stained sections, 11 cases were positive for Alpha Fetoprotein immunohistochemically, giving a sensitivity of 84%.

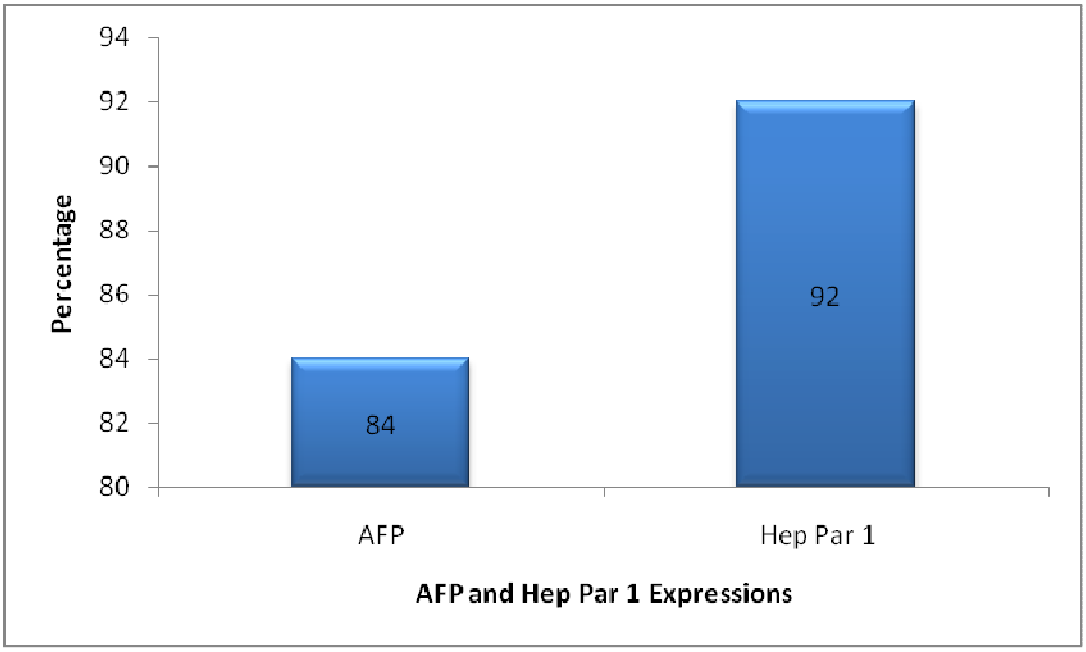
Hep Par 1 expression was also studied in all cases of hepatocellular carcinomas.

12 out of 13 cases were positive and only 1 case was negative; thus the sensitivity of Hep Par 1 in this study was found to be 92%.

So, it is observed that both Alpha Fetoprotein and Hep Par 1 are good immunohistochemical markers for the diagnosis of hepatocellular carcinoma; and Hep Par 1 is more sensitive than Alpha Fetoprotein.

CHART- 3

PERCENTAGE POSITIVITY OF ALPHA RETROPROTIEN (AFP) AND HEP PAR 1 EXPRESSIONS IN HEPATOCELLULAR CARCINOMA CASES



Among the 13 cases reported as metastatic carcinomas of liver, all cases were studied using the immunohistochemical markers Cytokeratin7 and Cytokeratin20.

TABLE 3

EXPRESSION OF CYTOKERATIN7 AND CYTOKERATIN20 IN METASTATIC CARCINOMAS

S.No.	MARKERS	No. OF POSITIVE CASES	No. OF NEGATIVE CASES	PERCENTAGE OF POSITIVE CASES (%)
1.	CYTOKERATIN7	7	6	54
2.	CYTOKERATIN20	3	10	23

From the study, the following observations were obtained:

No. of Cytokeratin7 positive and Cytokeratin20 negative cases: 7 (54%)

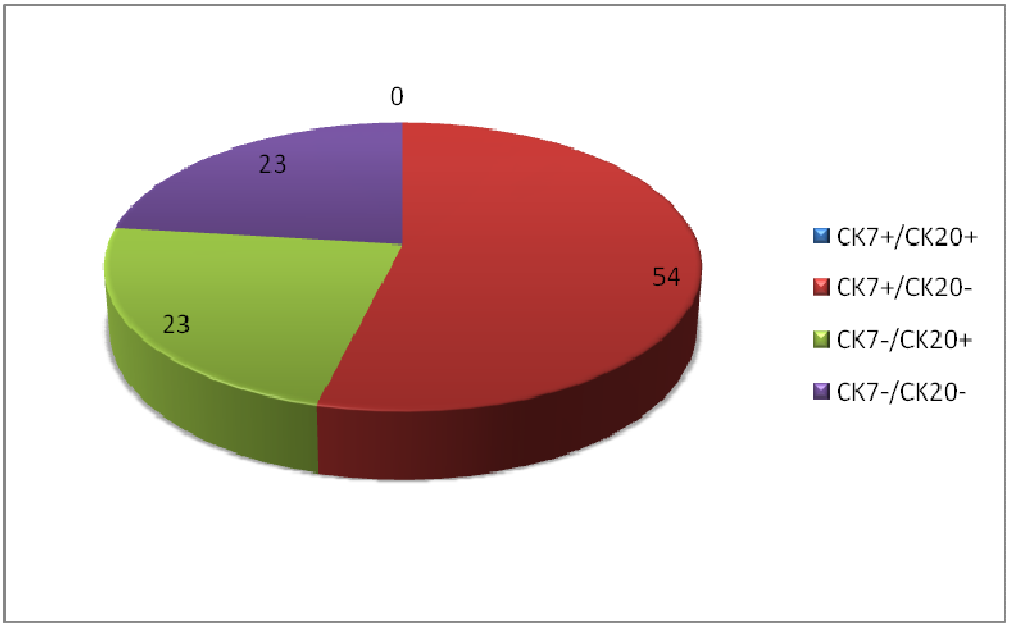
No. of Cytokeratin7 negative and Cytokeratin20 positive cases: 3 (23%)

No. of Cytokeratin7 negative and Cytokeratin20 negative cases: 3 (23%)

No. of Cytokeratin7 positive and Cytokeratin20 positive cases: 0 (0%)

CHART-4

EXPRESSION OF CYTOKERATIN7 AND CYTOKERATIN20 IN METASTATIC CARCINOMAS



Analysing the possible sites of primary tumour in these 13 cases of metastatic carcinomas :

TABLE 4

PATTERNS OF CYTOKERATIN EXPRESSION IN VARIOUS TYPES OF METASTATIC TUMOURS

CYTOKERATIN 7 POSITIVE/ CYTOKERATIN 20 POSITIVE TUMOURS	CYTOKERATIN 7 POSITIVE/ CYTOKERATIN 20 NEGATIVE TUMOURS
Urothelial carcinoma Pancreas Biliary tract Esophagus/Stomach Mucinous carcinoma (ovarian, colon, mucinous broncheoalveolar)	Breast Lungs Esophagus/Stomach Pancreas Biliary tract Ovary (nonmucinous) Endometrium
CYTOKERATIN 7 NEGATIVE/ CYTOKERATIN 20 POSITIVE TUMOURS	CYTOKERATIN 7 NEGATIVE/ CYTOKERATIN 20 NEGATIVE TUMOURS
Colorectal	Prostate Renal Cell Carcinoma Adrenal Cortical Carcinoma

In the study, the most common expression is cytokeratin 7 positive/ cytokeratin 20 negative expression which is observed in 54% of cases, followed by cytokeratin 7 negative/ cytokeratin 20 positive cases (23%), cytokeratin 7 negative/ cytokeratin 20 negative (23%) and cytokeratin 7 negative/ cytokeratin 20 negative (0%) expressions.

This leads to an observation that the most common primary sites of tumours to metastasize to the liver are esophagus, stomach, breast, lungs, pancreas, biliary tract, ovary and endometrium.

At this point, it is worth mentioning about the size of liver biopsy specimens. The size of the liver biopsy specimens received in the laboratory during the period of the study was less than 1.5 cm which did not meet the required adequacy (atleast 1.5 cm). So, only the more commonly used markers Alpha Fetoprotein, Hep Par 1, Cytokeratin 7 and Cytokeratin 20 could be studied.

Special stain with reticulin by Gomori's method was done in 10 difficult cases to differentiate benign from malignant lesions.

Reticulin fibres in the benign processes revealed one-cell thick liver plates; whereas in dysplastic and carcinomatous deposits, they

showed increased thickness of liver cell plates, which appeared to be more than two cell-thick , normal being one-cell thickness.

Colour Plates

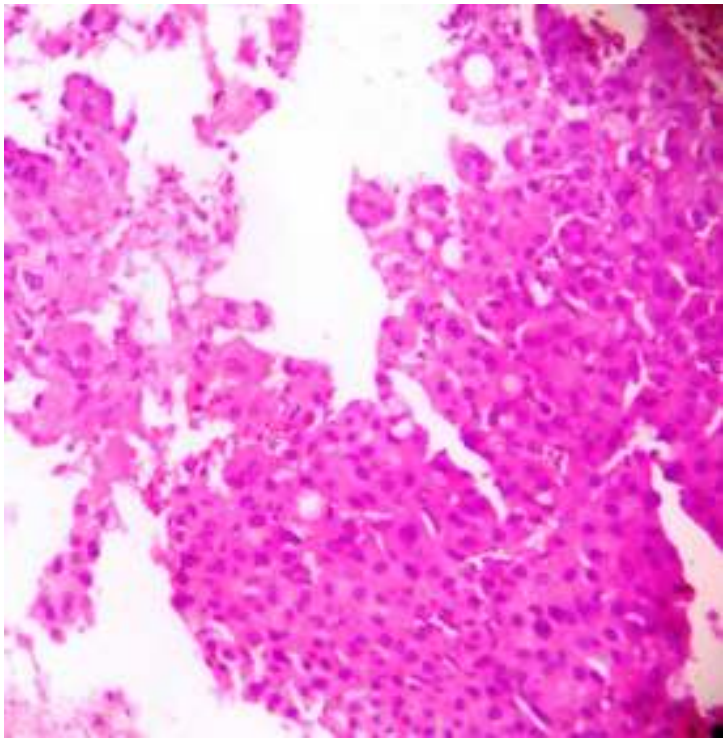


Fig. 1 : Low Power view showing well differentiated Hepatocellular Carcinoma composed of tumor cells arranged in 2- to 3- cell thick trabeculae (10x)

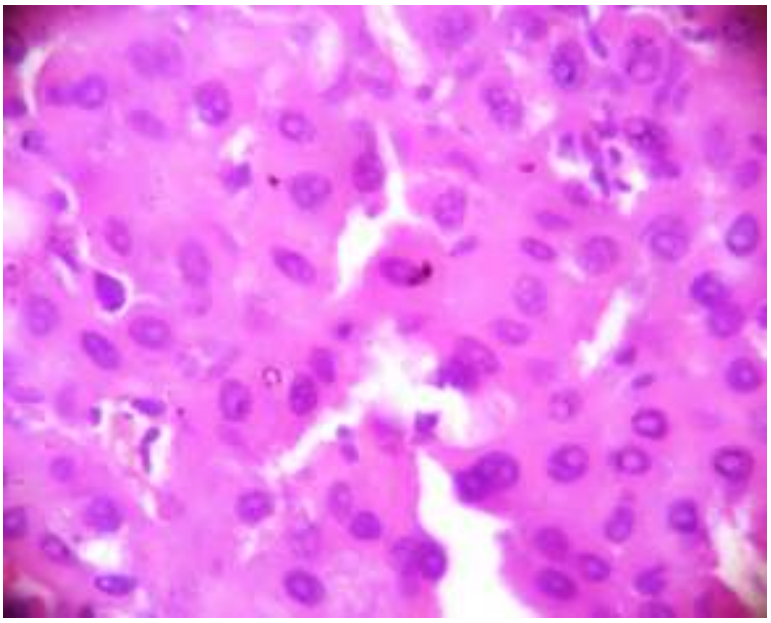


Fig. 2 : High Power view showing polygonal tumor cells with nuclear pleomorphism and high nuclear to cytoplasmic ratio (40x)

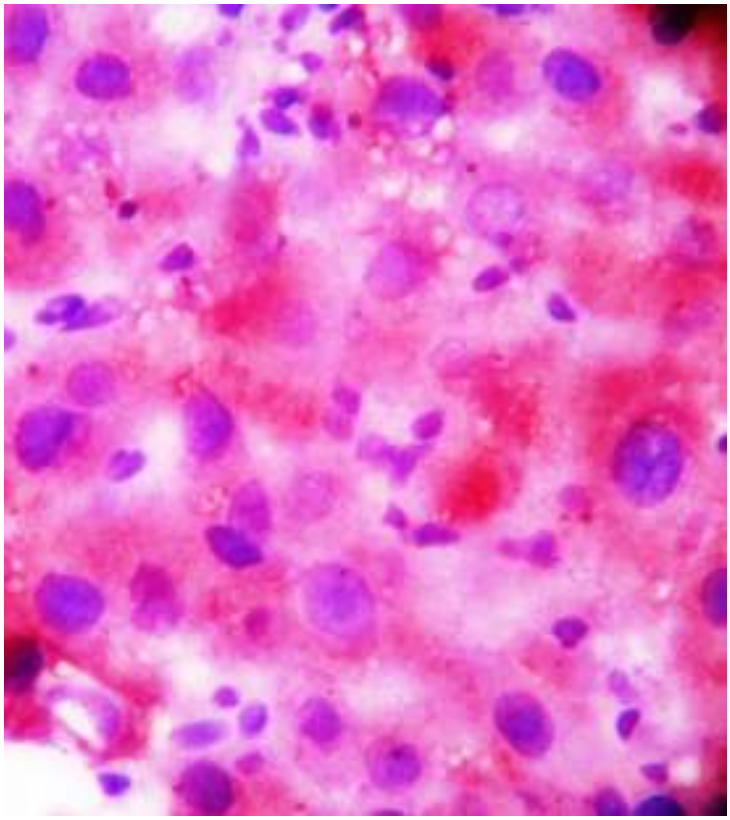


Fig. 3 : Hepatocellular Carcinoma showing tumor cells which are strongly positive for Alpha Feto-protein (40x)

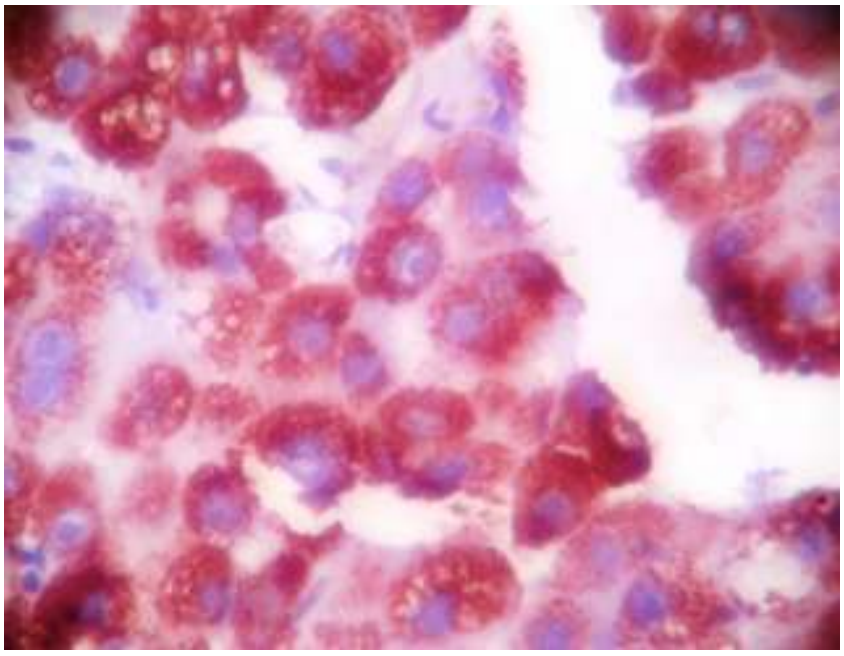


Fig. 4 : High power view showing cytoplasmic granular positivity of Hep par1 in the tumor cells (40x)

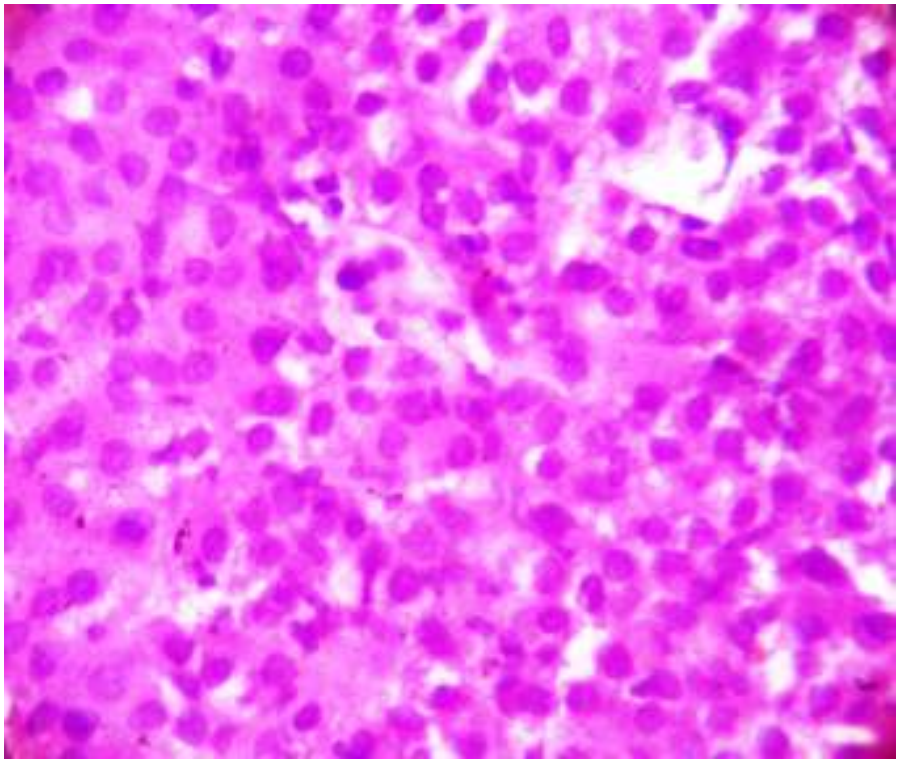


Fig. 5 : High power view showing sheets of tumor cells with moderate eosinophilic cytoplasm and nuclear atypia (40x)

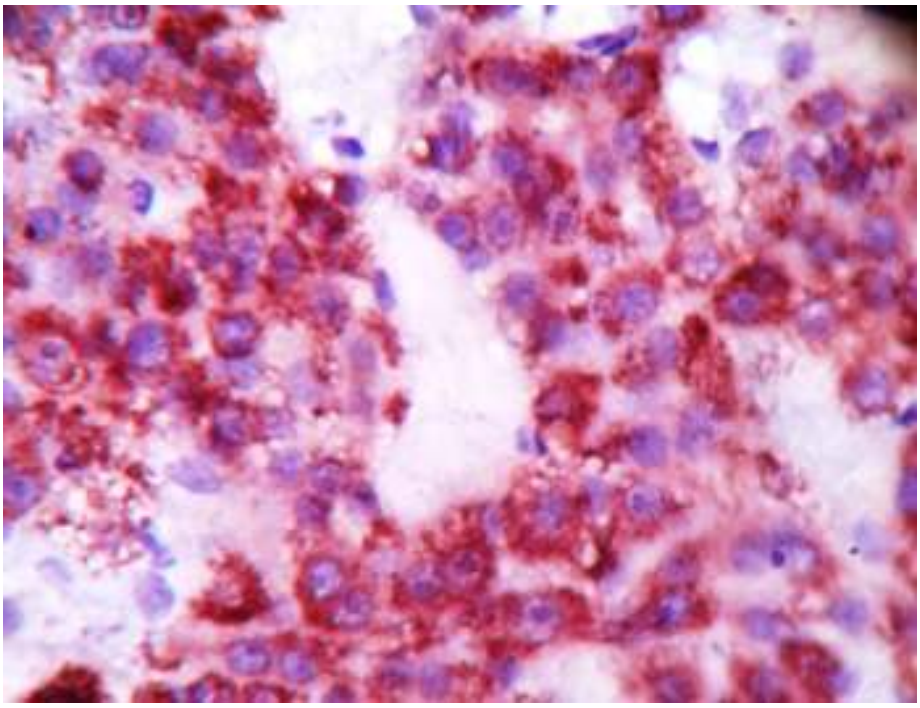


Fig. 6 : Immunohistochemical staining of hepatocellular carcinoma with Hep par1. The tumor cells are strongly positive for Hep par1 with a cytoplasmic granular pattern (40x)

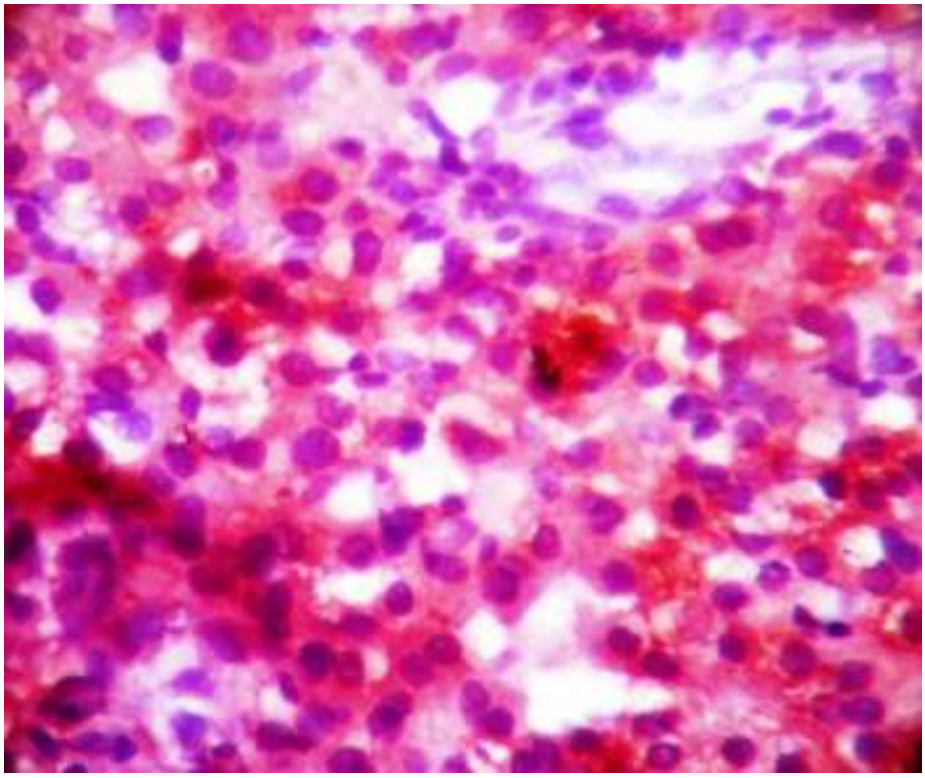


Fig. 7 : Immunohistochemical staining of hepatocellular carcinoma with Alpha Feto-protein. Most tumor cells express Alpha Feto-protein in the cytoplasm (40x)

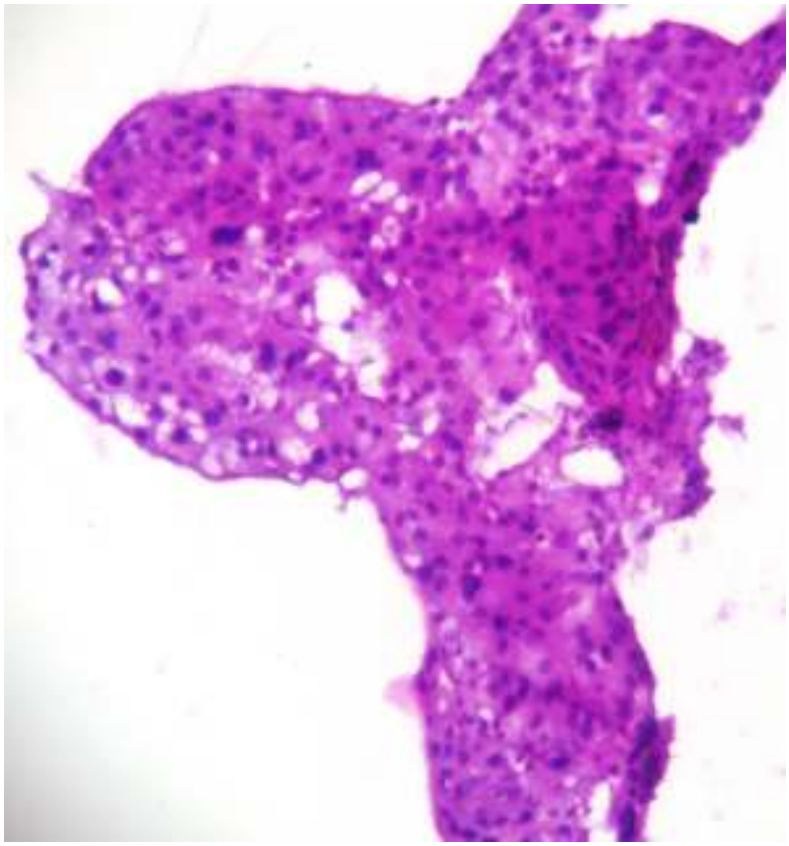


Fig. 8 : Low power view showing tumor cells of hepatocellular carcinoma (10x)

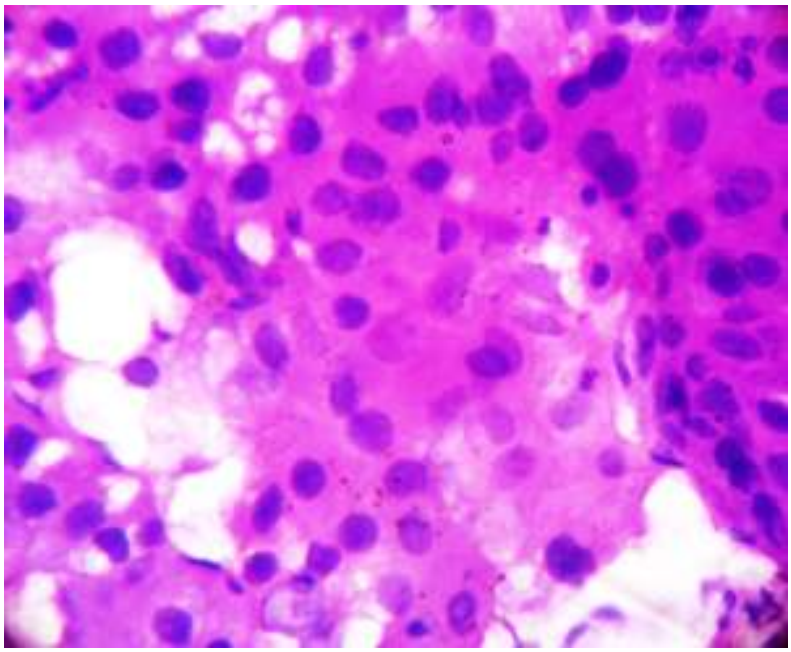


Fig. 9 : High power view of hepatocellular carcinoma with nuclear pleomorphism and atypia (40x)

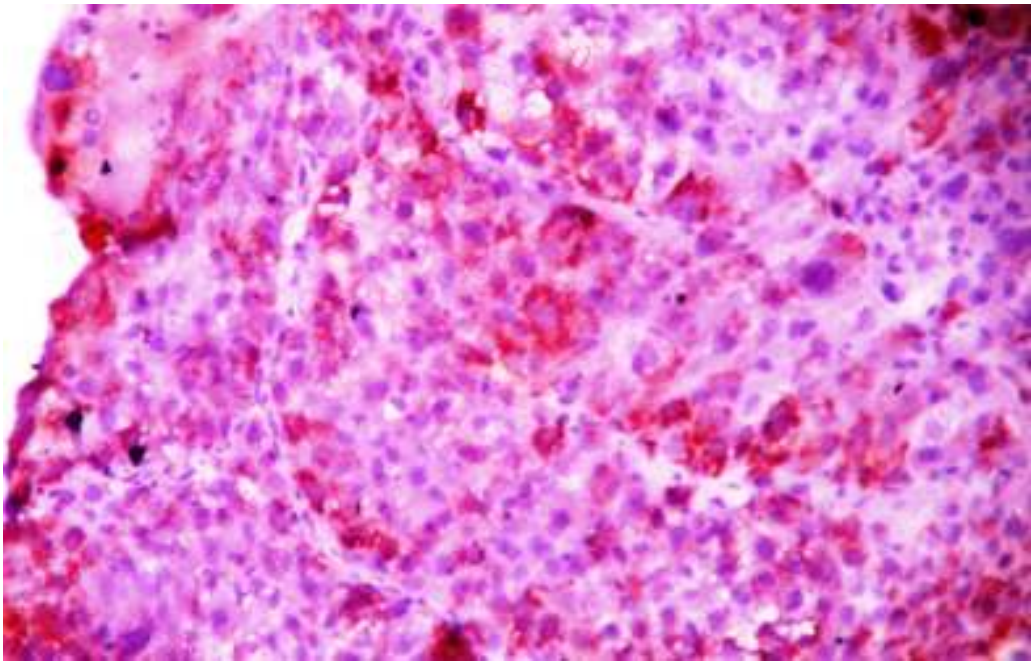


Fig. 10 : Immunohistochemical staining of hepatocellular carcinoma with Hep par1 shows a cytoplasmic granular pattern representing hepatocellular differentiation (10x)

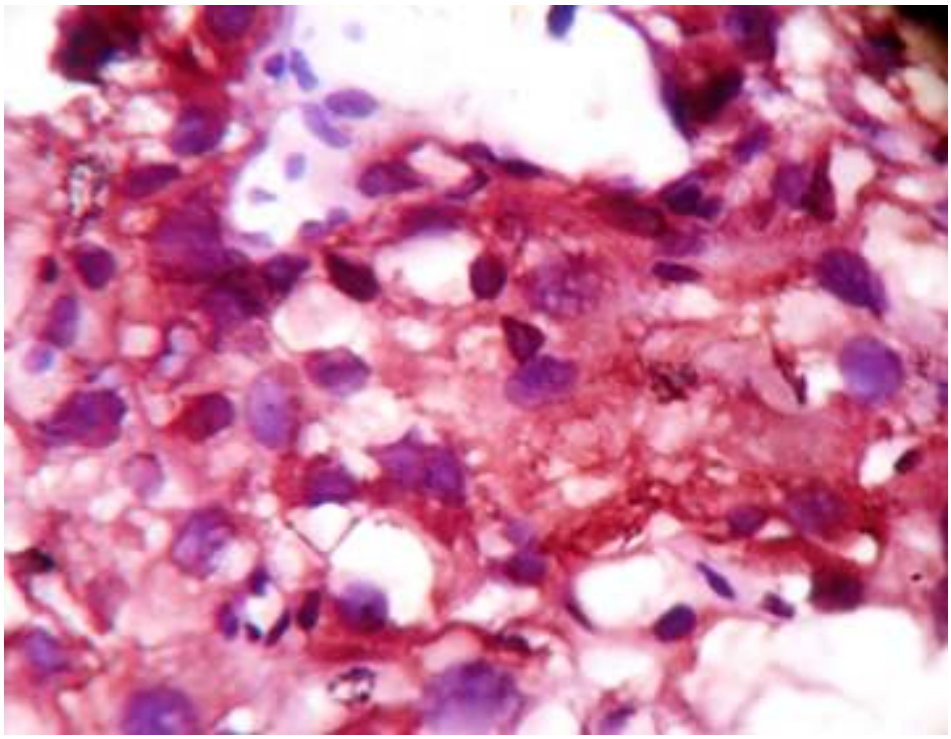


Fig. 11 : Tumor cells of hepatocellular carcinoma showing diffuse cytoplasmic positivity for Alpha Feto-protein (40x)

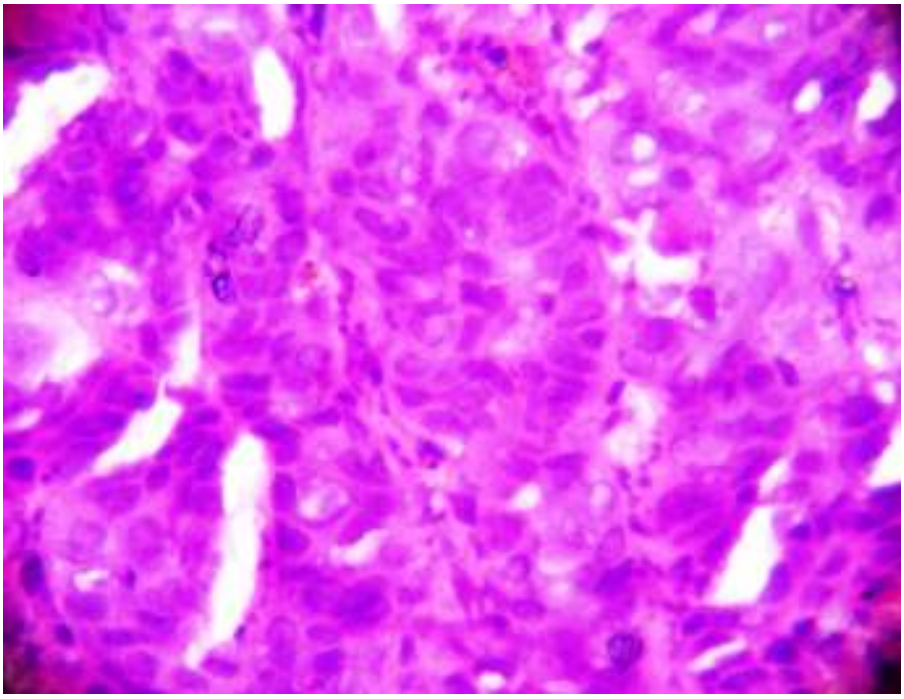


Fig. 12 : High power view of metastatic adenocarcinomatous deposits in liver showing malignant tumor cells arranged in glandular pattern (40x)

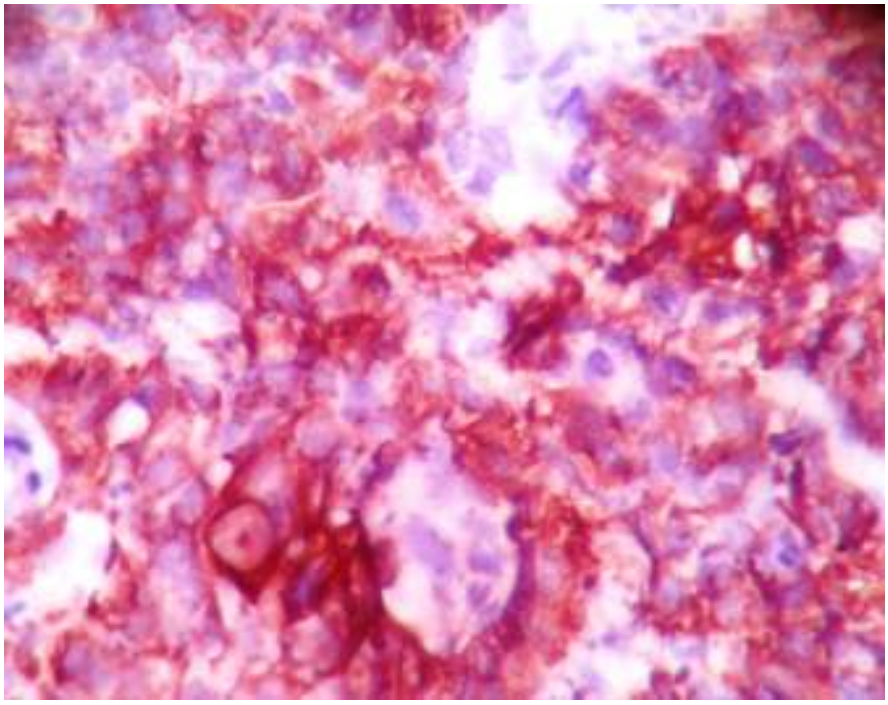


Fig. 13 : The tumor cell in metastatic deposits showing strong cytoplasmic positivity for Cytokeratin 7 (40x)

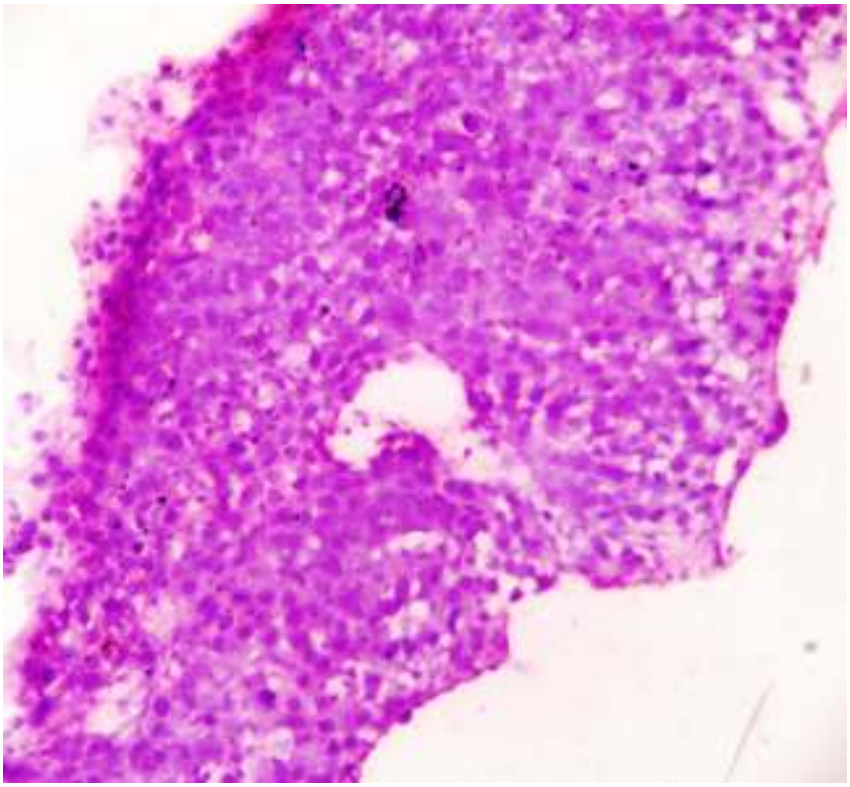


Fig. 14 : Hepatocellular carcinoma showing tumor cells with nuclear pleomorphism (10x)

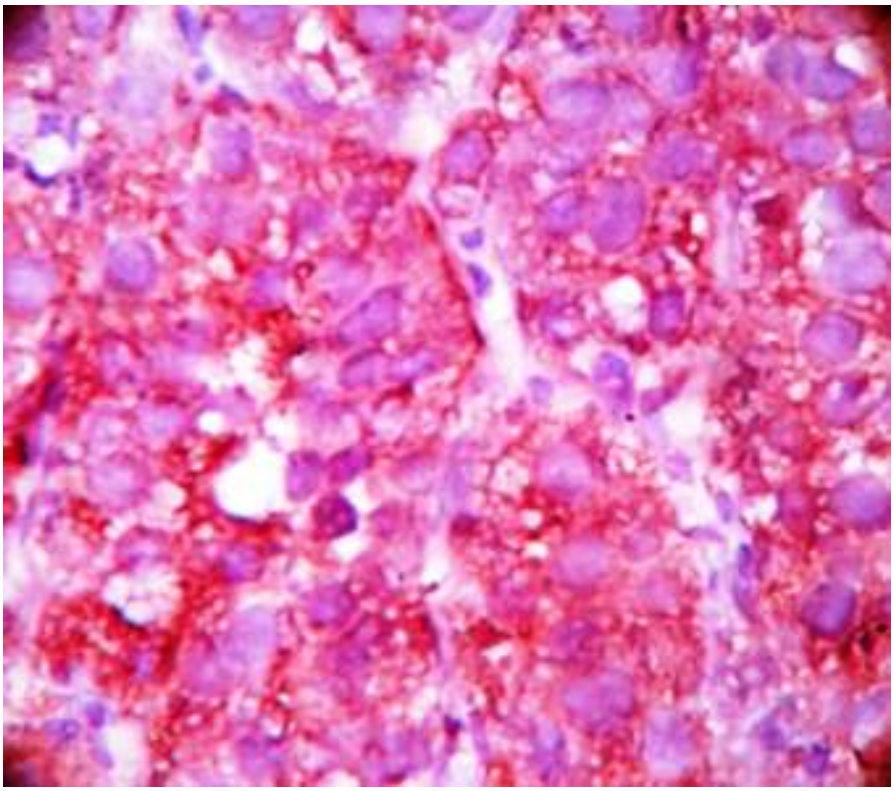


Fig. 15 : The tumor cells are strongly positive for Alph Feto-protein (40x)

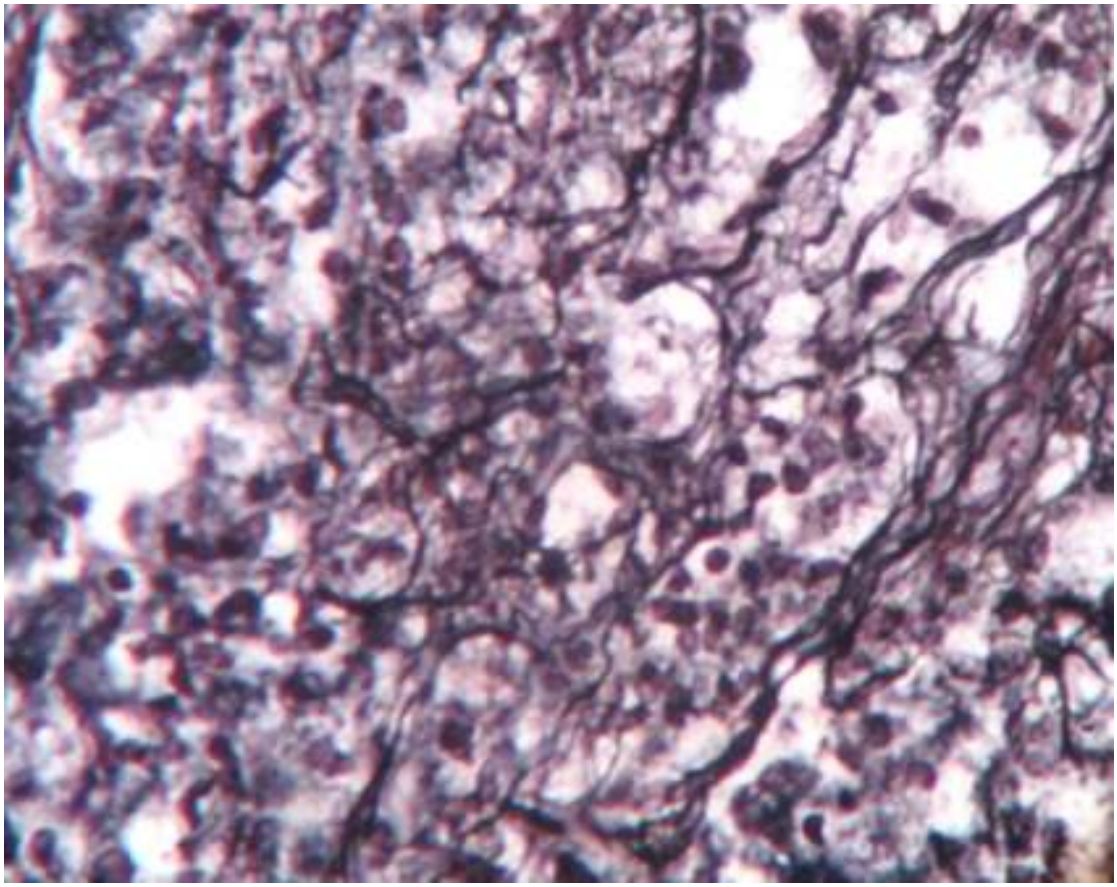


Fig. 16 : Well-differentiated hepatocellular carcinoma showing a compact pattern with inapparent sinusoids (Reticulin Stain) (40x)

Discussion

DISCUSSION

26 cases of hepatic malignancies during the period from July 2011 to July 2012 were studied.

Statistical data of percentage positivity of Alpha Fetoprotein and Hep Par 1 in primary hepatic malignancies and Cytokeratin 7 and Cytokeratin 20 in metastatic carcinomas of the liver were studied , and compared with those in the literature.

HEP PAR 1 EXPRESSION

According to a study by Lau SK et al in 2002[28], who evaluated Hep Par 1 in 42 cases of hepatocellular carcinomas, 38 cases (90%) were positive.

Zhen Fan et al in 2003[42] studied Hep Par 1 expression in 19 cases of hepatocellular carcinomas and found positivity in 18 cases (94%).

In a study by Pozharisski KM et al in 2008[46], 55 primary hepatic tumours were studied; Hep Par 1 was positive in all the cases (100%).

According to a study by Karabork A et al in 2010[51], Hep Par 1 was positive in 95.6% cases among 68 cases studied.

In the present study, among the 13 cases diagnosed as hepatocellular carcinoma, 12 cases (92%) were positive for Hep Par 1, which was comparable with the other studies.

TABLE-5

COMPARISON OF % POSITIVITY OF HEP PAR 1 IN DIFFERENT STUDIES

STUDY GROUP	YEAR	No. OF CASES STUDIED	% POSITIVITY OF HEP PAR 1
Lau SK et al	2002	42	90
Zhen Fan et al	2003	19	94
Pozharisski et al	2008	55	100
Karabork et al	2010	68	95.6
Present Study	2011	26	92

Hence it is observed that Hep Par 1 is a useful marker in the diagnosis of hepatocellular carcinoma.

ALPHA FETOPROTEIN EXPRESSION

Porcell et al in 2000[41] studied 57 previously characterized hepatic neoplasms, 13 cases were hepatocellular carcinomas. Alpha Fetoprotein was detected in 4 of these 13 cases (30%).

In a study Lau SK Prakash S et al in 2002[28], 42 cases of hepatocellular carcinomas were studied using a panel of markers including Alpha Fetoprotein, Hep Par 1, p CEA, CD10 and villin. They found that Alpha Fetoprotein was observed in one third of the cases.

According to a study by Ali S. Sawan et al in 2009[38], 41 cases histologically diagnosed liver biopsies were studied including hepatocellular carcinoma, cholangiocarcinoma and metastatic carcinoma; Alpha Fetoprotein was positive in 43.7% of hepatocellular carcinoma cases.

In the present study, 11 out of 13 cases of hepatocellular carcinomas stained positive for Alpha Fetoprotein; its sensitivity being 84% which is more than that found in other studies.

TABLE 6

COMPARISON OF % POSITIVITY OF ALPHA FETOPROTEIN EXPRESSION WITH OTHER STUDIES

STUDY GROUP	YEAR	No. OF CASES STUDIED	% POSITIVITY OF ALPHAFETO PROTEIN
Porcell et al	2000	13	30
Lau SK Prakash	2002	42	33
Ali S.Sawan	2009	41	43.7
Present Study	2011	26	84

Thus, Hep Par 1 is more sensitive than Alpha Fetoprotein in the diagnosis of Hepatocellular carcinoma.

CYTOKERATIN 7 AND CYTOKERATIN 20 EXPRESSIONS IN METASTATIC CARCINOMA:

Rullier A et al in 2000[59] studied 31 cases of colorectal carcinomas and cholangiocarcinomas. Cytokeratin 20 was positive in all cases of colorectal carcinomas.

Cholangiocarcinomas were positive for cytokeratin 7 and cytokeratin 20 in 96% and 70% cases respectively.

In a study by Ali S. Sawan et al in 2009[38], which included 41 cases of histologically diagnosed liver biopsies including primary

liver malignancies, cholangiocarcinoma and secondaries in the hepatic parenchyma, Cytokeratin 7 positive and cytokeratin 20 negative expression were identified in 100 percent of cholangiocarcinomas, one metastatic pancreatic carcinoma and all metastatic gastric carcinomas. It was also found that cytokeratin 7 negative and cytokeratin 20 positive expression indicates colorectal carcinoma metastatic to the liver and that cytokeratin 7 expression excludes hepatocellular carcinoma.

In the present study, cytokeratin 7 and cytokeratin 20 were positive in 7 and 3 cases respectively in the 13 metastatic carcinomas studied. Also, cytokeratin 7 positive/ cytokeratin 20 negative expression is the most common observation (54%) in this study which indicates that that the most common tumours to metastasize to the liver are from esophagus, stomach, biliary tract, pancreas, breast, lungs, ovary and endometrium.

Ideally a panel of markers including CD10, p CEA, Cytokeratin 19, MOC-31 and villin should be used to diagnose hepatocellular carcinoma and to assess the possible location of the origin the metastatic tumours with more accuracy; but only the more common and reliable markers Alpha Fetoprotein, Hep Par 1, Cytokeratin 7 and Cytokeratin 20 were included in the present study.

This is because of the availability of very limited tissue in the biopsy specimen which warranted the judicious use of markers.

Special stain study with reticulin by Gomori's method was also done. It was found to be useful in differentiating benign from malignant lesions of liver.

Summary

SUMMARY

This is a prospective study on hepatic neoplasms reported on liver biopsy specimens received in the Department of Pathology, Coimbatore Medical College Hospital, Coimbatore over a period of one year from July 2011 to July 2012.

26 out of 34 liver biopsy specimens received were reported as hepatic neoplasms; 13 cases were hepatocellular carcinomas and 13 were metastatic tumours in the liver.

All 13 cases of hepatocellular carcinomas were studied using the immunohistochemical markers Alpha Fetoprotein and Hep Par 1. Alpha Fetoprotein was positive in 11 cases (84%) and Hep Par 1 was positive in 12 cases (92%).

Cytokeratin 7 and Cytokeratin 20 were studied in 13 cases of metastatic tumours.

Majority of tumours showed Cytokeratin 7 positive/ cytokeratin 20 negative expression (54%), which indicates that the most common primary sites were esophagus, stomach, pancreas, biliary tract, breast, lungs, ovary and endometrium.

If adequate liver biopsy specimen is received (atleast 1.5 cm), an expanded panel of markers can be used to identify the primary site of tumour with more accuracy.

Reticulin stain was also done in difficult cases to differentiate benign from malignant lesions of the liver, which was found to be useful.

Conclusion

CONCLUSION

Liver biopsy is an essential investigational procedure done for evaluation and management of patients with reference to the histological assessment of the liver. The liver disease can be diagnosed by the widely available, sensitive and relatively accurate blood investigations. But the valuable diagnostic tool which remain as gold standard in the liver biopsy.

An adequate sample measures 1.5 cm long and 1.2 to 2mm in thickness. Histologically, it should contain atleast 6 to 8 portal triads.

The distinction of hepatocellular carcinoma and other neoplasms involving the liver can be at times difficult and challenging. Immunohistochemistry is useful in such cases especially to differentiate primary from matastatic hepatic neoplasms.

The immunohistochemical markers Alpha Fetoprotein and Hep Par 1 usually indicate malignancy in hepatocellular nodule and hepatocytic histogenesis of a malignancy. Hep Par1 is a more sensitive marker than Alpha Fetoprotein.

Cytokeratin 7 and Cytokeratin 20 are the markers to assess the probable sites of tumours in cases of metastasis. From this study,

the most common sites of primary tumours are esophagus, stomach,pancreas, breast, biliary tract, ovary and endometrium.

If adequate tissue is available, an additional panel of markers can be used including Glypican 3, CD10, pCEA, Cytokeratin 19, villin to identify the primary site of tumour with more accuracy.

Appendices

ANNEXURE

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

COIMBATORE

Particulars of the Patient :

Name	:	Hospital	:
Case No.	:	Date	:
Age/Sex	:	I.P. No.	:
Address	:	Ward No.	:
Occupation	:	Religion	:

Presenting Complaints and Duration :

Abdominal Pain +/-

Nausea / Vomiting +/-

Fever +/-

Loss of Appetite / Loss of Weight +/-

Bleeding Diathesis +/-

Past History :

Previous History of Hepatitis infection +/-

Transfusions +/-

Drug History +/-

Chronic Diseases +/-

Family History :

Recurrent Jaundice +/-

Malignancy +/-

Personal History :

Diet / Appetite / Bowel and Bladder Habits

Sleep / Alcohol Intake

Menstrual History :

General Physical Examination :

Built	:	Febrile / Afebrile	:
Nourishment	:	Pallor	:
Conscious	:	Jaundice	:
Weight	:	Cyanosis	:
Pulse	:	Clubbing	:
Respiratory Rate	:	Lymphadenopathy	:
Pulse Rate	:	Edema	:

Systemic Examination :

P/A : Hepatomegaly

Splenomegaly

Ascites

CVS : **RS :** **CNS :** **Musculo Skeletal System :**

Clinical Diagnosis :

Investigations :

1) Complete Hemogram
2) Liver Function Tests
3) USG / CT Abdomen
4) Upper / Lower GI Endoscopy

Liver Biopsy Evaluation :

Histopathological Diagnosis :

Immunohistochemistry :

Alpha Fetoprotein +/-

Hep par1 +/-

Cytokeratin 7 +/-

Cytokeratin 20 +/-

Final Diagnosis :

Primary or secondary neoplasm of liver.

If secondary malignancy – possible site of primary

Bibliography

BIBLIOGRAPHY

1) Di Bisceglie AM, Carithers RL Jr, Gores GJ. Hepatocellular Carcinoma. Hepatology. 1998: 28(4): 1161-5.

2) Abbruzzese JL, Abbruzzese MC, Lenzi R, Hess KR, Raber MN. Analysis of a diagnostic strategy for patients with suspected tumors of unknown origin. J Clin Onc. 1995;13:2094–2103.

3) Chu P, Wu E, Weiss LM. Cytokeratin 7 and Cytokeratin 20 expression in epithelial neoplasms: A survey of 435 cases. Mod Pathol. 2000;13:962–972.

4) Goldstein NS, Thomas M. Mucinous and nonmucinous bronchioloalveolar adenocarcinomas have distinct staining patterns with thyroid transcription factor and cytokeratin 20 antibodies. Am J Clin Pathol. 2001;116:319–325.

5) Barbara A. Centeno, M.D. Pathology of Liver Metastases. 2006; vol.13; 1-23.

6) Muhammad Tahir Khadim, Shahid Jamal et al. Diagnostic challenges and role of immunohistochemistry in metastatic Liver Disease. Asian Pacific J Cancer Prev; vol.12: 373-76.

7) Hammar SP. Metastatic adenocarcinoma of unknown primary origin. Hum Pathol. 1998;29:1393–1402.

8) Harlamert HA, Mira J, Bejarano PA, Baughman RP, Miler MA, Whitsett JA, Yassin R. Thyroid transcription factor-1 and

cytokeratins 7 and 20 in pulmonary and breast carcinoma. Acta Cytol. 1998;42:1382–1388.

9) Lagendijk JH, Mullink H, Van Diest PJ, Meijer GA, Meijer CJLM. Tracing the origin of adenocarcinomas with unknown primary using immunohistochemistry. Hum Pathol. 1998;29:491–497.

10) Siegfried, M.Aabilitationss Chriff.Leipzig: F.A.Brockhaus.cited in Glegg. Some carbohydrate components of Reticular Fibres. Science 118; 614-16.

11) Strum, Judy M., Gartner,Leslie P;Hiatt, James L. Cell Biology and Histology. Hagerstwon, MD: Lippincott Williams and Wilkins; p.83. ISBN0-7817-8577-4.

12) Lagendijk JH, Mullink H, Van Diest PJ, Meijer GA, Meijer CJLM. Immunohistochemical differentiation between primary adeno-carcinomas of the ovary and ovarian metastases of colonic and breast origin. J Clin Path. 1999;52:283–290.

13) Loy TS, Calaluce RD. Utility of cytokeratin immunostaining in separating pulmonary adenocarcinomas from colon adeno-carcinomas. Am J Clin Pathol. 1994;102:764–767.

14) Raab SS, Berg LC, Swanson PE, Wick MR. Adenocarcinoma in the lung in patients with breast cancer. Am J Clin Pathol. 1993;100:27–35.

15) Rubin BP, Skarin AT, Pisick E, Rizk M, Salgia R. Use of cytokeratin 7 and 20 in determining the origin of metastatic carcinoma of unknown primary, with special emphasis on lung cancer. Eur J Cancer Prev. 2001;10:77–82.

16) Tan J, Sidhu G, Greco MA, Ballard H, Wieczorek R. Villin, cytokeratin 7 and cytokeratin 20 expression in pulmonary adenocarcinoma with ultrastructural evidence of microvilli with rootlets. Hum Pathol. 1998;29:390–396.

17) Tot T. Adenocarcinomas metastatic to the liver. Cancer. 1999;85:171–177.

18) Wang NP, Zee S, Zarbo RJ, Bacchi CE, Gown AM. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. Appl Immunohistochem. 1995;3:99–107.

19) Wauters CCAP, Smedts F, Gerrits LGM, Bosman FT, Ramaekers FCS. Keratins 7 and 20 as diagnostic markers of carcinomas metastatic to the ovary. Hum Pathol. 1995;26:852–855.

20) Nystrom JS, Weiner JM, Heffelfinger-Juttner J, Irwin LE, Bateman JR, Wolf RM. Metastatic and histologic presentations in unknown primary cancer. Semin Oncol 1977;4:53–8.

21) Hillen HFP. Unknown primary tumours. Postgrad Med J 2000;76:690–3.

22) Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. Cancer 2004;100:1776–85.

23) Pavlidis N, Briasoulis E, Hainsworth J, Greco FA. Diagnostic and therapeutic management of cancer of an unknown primary. Eur J Cancer 2003;39:1990- 2005.

24) Sheahan K, O'Keane JC, Abramowitz A, et al. Metastatic adenocarcinoma of an unknown primary site. A comparison of the

relative contributions of morphology, essential clinical data and CEA immunostaining status. Am J Clin Pathol 1993;99:729-35.

25) Sanjay Kakar, MD; Allen M.Gown, MD et al. Best Practices in Diagnostic Immunohistochemistry. Arch Pathol Lab Med 2007; vol.131;1648-54.

26) Jaffe ES. Hematopathology: integration of morphologic features and biologic markers for diagnosis. Mod Pathol 1999;12:109–15.

27) DeYoung BR, Wick MR. Immunohistologic evaluation of metastatic carcinomas of unknown origin: an algorithmic approach. Semin Diagn Pathol 2000;17:184–93.

28) Lau SK, Prakash S,Geller, MD et al. Comparative immunohisto-chemical profile of Hepatocellular Carcinoma, cholangiocarcinoma and metastatic adenocarcinoma.Human Pathol.2002; 33(12); 1175-81.

29) Lagendijk JH, Mullink H, van Diest PJ, Meijer GA, Meijer CJ. Immunohistochemical differentiation between primary adenocarcinomas of the ovary and ovarian metastases of colonic and breast origin. Comparison between statistical and an intuitive approach. J Clin Pathol 1999;52:283–90.

30) Ma CK, Zarbo RJ, Frierson H F Jr, Lee MW. Comparative Immunohistochemical study of primary and metastatic carcinoma of the liver. American J Clin Pathol. 1993 99(5); 551-7.

31) Pecciarini L, Giulia Cangi M, Doglioni C. Identifying the primary sites of metastatic carcinoma: the increasing role of immunohistochemistry. Curr Diagn Pathol 2001;7:168–75.

32) Hammar SP. Metastatic adenocarcinoma of unknown primary origin. Hum Pathol 1998;29:1393–402.

33) Kaufmann O, Dietel M. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinomas and large cell carcinomas compared to surfactant proteins A and B. Histopathology 2000;36:8.

34) Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. Cancer 1999;85:171–7.

35) Dennis JL, Vass JK, Wit EC, Keith WN, Oien KA. Identification from public data of molecular markers of adenocarcinoma characteristic of the site of origin. CancerRes 2002;62:5999–6005.

36) Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844–7.

37) Perry A, Parisi JE, Kurtin PJ. Metastatic adenocarcinoma to the brain: an immunohistochemical approach. Hum Pathol 1997;28:938–43.

38) Ali S. Sawan. The Diagnostic value of immunohistochemistry in the diagnosis of primary and secondary hepatic carcinomas. JKAU: Med.Sci., vol.16 No.4: 37-48.

39) Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical surveyof 476 primary and metastatic carcinomas. Am J Surg Pathol 2003;27:303–10.

40) Machado JC, Nogueira AM, Carneiro F, Reis CA, Sobrinho-Simoes M. Gastric carcinoma exhibits distinct types of cell differentiation: an immunohistochemical study of trefoil peptides (TFF1 and TFF2) and mucins (MUC1, MUC2, MUC5AC, and MUC6). JPathol 2000;190:437–43.

41) Porcell Al, De Young BR, Proca DM, Frankel WL. Immunohistochemical Analysis of hepatocellular carcinoma and adenocarcinoma of the liver. Mod Pathol.2000: 13(7): 773-8.

42) Zhen Fan M.D et al. Hep Par 1 Antibody stain for the differential diagnosis of hepatocellular carcinoma. Mod Pathol 2003: 16(2); 137-144.

43) Lagendijk JH, Mullink H, Van Diest PJ, Meijer GA, Meijer CJ. Tracing the origin of adenocarcinomas with unknown primary using immunohistochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. Hum Pathol 1998;29:491–7.

44) Kaufmann O, Deidesheimer T, Muehlenberg M, Deicke P, Dietel M.Immunohistochemical differentiation of metastatic breast carcinomas from metastatic adenocarcinomas of other common primary sites. Histopathology 1996;29:233–40.

45) Loy TS, Quesenberry JT, Sharp SC. Distribution of CA 125 in adenocarcinomas. An immunohistochemical study of 481 cases. Am J Clin Pathol1992;98:175–9.

46) Pozharisski KM, Granov DA et al. The significance of immunohistochemistry in the investigation of liver neoplasms, differential diagnosis and prognostic markers. Vopr Onkol.2008:54(4); 417-33.

47) Frierson HF Jr, Moskaluk CA, Powell SM, et al. Large-scale molecular and tissue microarray analysis of mesothelin expression in common human carcinomas. Hum Pathol 2003;34:605–9.

48) Ordonez NG. Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 2003;27:1418–28.

49) Wick MR, Lillemoe TJ, Copland GT, Swanson PE, Manivel JC, Kiang DT. Gross cystic disease fluid protein-15 as a marker for breast cancer: Immunohistochemical analysis of 690 human neoplasms and comparison with α-lactalbumin. Hum Pathol 1989;20:281–7.

50) Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF Jr. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. Mod Pathol 2003;16:913–9.

51) Karahork A, Kaygusuz G, Ekinci C. The Best immunohistochemical panel for diferntiating hepatocellular carcinoma from metastatic adenocarcinoma. Pathol Res Pract.2010: 206(8); 572-7.

52) Brown RW, Campagna LB, Dunn JK, Cagle PT. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. Am J Clin Pathol 1997;107:129.

53) Zamecnik J, Kodet R. Value of thyroid transcription factor-1 and surfactant apoprotein A in the differential diagnosis of pulmonary carcinomas: a study of 109 cases. Virchows Arch 2002;440:353–61.

54) Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Lab Invest 2000;80:1943–9.

55) Doughty SE, Ferrier RK, Hillan KJ, Jackson DG. The effects of ZENECA ZD8731, an angiotensin II antagonist, on renin expression by juxtaglomerular cells in the rat: comparison of protein and mRNA expression as detected by immunohistochemistry and in situ hybridization. Toxicol Pathol 1995;23:256-61.

56) Hsu FD, Nielsen TO, Alkushi A, et al. Tissue microarrays are an effective quality assurance tool for diagnostic immunohisto-chemistry. Mod Pathol 2002;15:1374–80.

57) Swierczynski SL, Maitra A, Abraham SC, et al. Analysis of novel tumor markers in pancreatic and biliary carcinomas using tissue microarrays. HumPathol 2004;35:357–66.

58) Hvidsten TR, Laegreid A, Komorowski J. Learning rule-based models of biological process from gene expression time profiles using gene onology. Bioinformatics 2003;19:1116–23.

59) Rullier A, Le Bail B, Fawaz R et al. Cytokeratin 7 and 20 expression in cholangiocarcinoma varies along the biliary tract but still differs from that in colorectal carcinoma metastasis. Am J Surg Pathol.2000; 24(6):870-6.

60) Tenti P, Aguzzi A, Riva C, et al. Ovarian mucinous tumors frequently express markers of gastric, intestinal, and pancreatobiliary epithelial cells. Cancer 1992;69:2131–42.

61) Ellis IO, Hitchcock A. Tumour marker immunoreactivity in adenocarcinoma. J Clin Pathol 1988;41:1064–7.

62) Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma. Eur J Cancer 2002;38:758–63.

63) Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000;13:962–72.

64) Park SY, Kim HS, Hong EK, Kim WH. Expression of cytokeratins 7 and 20 in primary carcinomas of the stomach and colorectum and their value in the differential diagnosis of metastatic carcinomas to the ovary. Hum Pathol 2002;33:1078–85.

65) Goldstein NS, Bassi D. Cytokeratins 7, 17, and 20 reactivity in pancreatic and ampulla of vater adenocarcinomas. Percentage of positivity and distribution is affected by the cut-point threshold. Am J Clin Pathol 2001;115:695–702.

66) Mintzer DM, Warhol M, Martin AM, Greene G. Cancer of unknown primary: changing approaches. A multidisciplinary case presentation from the Joan Karnell cancer center of Pennsylvania hospital. Oncologist 2004;9:330–8.

67) Bergman S, Graeme- Cook F, Pitman MB. The usefulness of Reticulin stain in the differential diagnosis of liver nodules on fine needle aspiration biopsy cell block preparations. Mod Pathol. 1997; 10(12): 1258-64.

MASTER CHART

S.NO.	BIOPSY NO.	IP NO.	AGE	SEX	CLINICAL DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	IMMUNO HISTOCHEMISTRY				FINAL DIAGNOSIS
							AFP	HEP PAR-1	CYTOKERATIN 7	CYTO KERATIN 20	
1.	240/10	2353	48	M	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
2.	1615/10	42758	54	M	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	-	+	Metastatic Adenocarcinomatous Deposits
3.	2448/10	68472	59	F	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	-	-	-	-	Metastatic Adenocarcinomatous Deposits
4.	2495/10	60665	78	M	? Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
5.	1019/11	29392	60	M	? Hepatoma	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
6.	1198/11	30736	49	F	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
7.	1378/11	33429	58	F	? Hepatoma	Hepatocellular Carcinoma	+	-	TI	TI	Hepatocellular Carcinoma
8.	1444/11	52787	68	M	Secondaries Liver	Hepatocellular Carcinoma	+	-	TI	TI	Hepatocellular Carcinoma
9.	2331/11	53587	40	M	? Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
10.	3479/11	61487	45	M	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
11.	4562/11	65432	65	M	Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
12.	4641/11	68716	62	M	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	-	-	-	-	Metastatic Adenocarcinomatous Deposits

13.	5308/11	70352	55	M	? Hepatoma	Hepatocellular Carcinoma	-	+	TI	TI	Hepatocellular Carcinoma
14.	5720/11	71487	45	M	? Hepatoma	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
15.	5855/11	73612	70	M	? Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	-	+	Metastatic Adenocarcinomatous Deposits
16.	236/12	3406	67	M	? Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
17.	554/12	5402	48	F	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	-	-	Metastatic Adenocarcinomatous Deposits
18.	604/12	9727	50	M	Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
19.	688/12	13156	70	M	? Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
20.	755/12	14325	40	M	? Hepatoma	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
21.	1005/12	21258	60	M	? Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
22.	1035/12	22217	71	M	? Hepatoma	Metastatic Adenocarcinomatous Deposits	TI	TI	+	-	Metastatic Adenocarcinomatous Deposits
23.	1198/12	25289	78	M	Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
24.	1489/12	29315	59	M	Secondaries Liver	Hepatocellular Carcinoma	-	+	TI	TI	Hepatocellular Carcinoma
25.	1495/12	29360	68	M	Hepatoma	Hepatocellular Carcinoma	+	+	TI	TI	Hepatocellular Carcinoma
26.	472/12	18903	52	F	Secondaries Liver	Metastatic Adenocarcinomatous Deposits	TI	TI	-	+	Metastatic Adenocarcinomatous Deposits

TI – Tissue Insufficient

ABSTRACT

Hepatocellular carcinoma is the fifth most common malignancy in the world. Liver is also a common site for metastatic tumours from various solid organs. This is a study of 26 cases of liver biopsies received in the Department of Pathology, Coimbatore Medical College Hospital, Coimbatore over a period from July 2011 to September 2012. Immunohistochemical markers Alpha Fetoprotein and Hep Par 1 were used to confirm the diagnosis of hepatocellular carcinoma; Alpha Fetoprotein was positive in 11 out of 13 cases of hepatocellular carcinoma(84%) and Hep Par 1 was positive in 12 cases(92%). The markers Cytokeratin 7 and Cytokeratin 20 were helpful in secondary tumours to assess the possible site of primary. No. of cases of CK7 CK 20 , CK7 CK 20 , CK7 CK20 ,CK7 CK20 were found to be 54%, 23%, 23% and 0% respectively. Reticulin stain was also found to be useful in difficult cases to differentiate benign lesions of liver from well-differentiated hepatocellular carcinoma.

KEYWORDS: Hepatocellular carcinoma, Alpha Fetoprotein, Hep Par 1, Cytokeratin 7, Cytokertain 20, Reticulin